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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	10/038722
Filing Date	January 8, 2002
First Named Inventor	Robert C. Ladner
Art Unit	1652
Examiner Name	Moore, William W.
Attorney Docket Number	D0617.70005US01

Total Number of Pages in This Submission: 5

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
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- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- [ ] Proprietary Information
- [ ] Status Letter
- [x] **Other Enclosure(s) (please identify below):**

1. Part B – Issue Fee Transmittal (PTOL-85 Form), 1 page
2. Application for Patent Term Adjustment, 3 pages
3. Check in the amount of $1,900
4. Return Receipt Postcard

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

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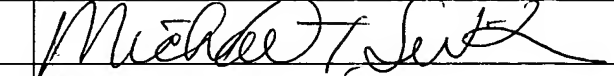
Signature			
Printed name	Michael T. Siekman		
Date	September 16, 2005	Reg. No.	36,276

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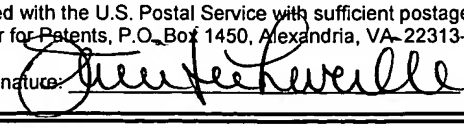
Dated: September 16, 2005 Signature:  (Jennifer Leveille)

DOCKET NO.: D0617.70005US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Arthur Charles Ley et al.
Serial No.:	10/038722
Confirmation No.:	4070
Filed:	January 8, 2002
For:	ITI-D1 KUNITZ DOMAIN MUTANTS AS hNE INHIBITORS
Examiner:	William W. Moore
Art Unit:	1652

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to **MS Issue Fee**, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date indicated below.

Dated: September 16, 2005

Jennifer Leveille

MS Issue Fee
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR PATENT TERM ADJUSTMENT UNDER 37 CFR § 1.705

Sir:

Applicants file this Application for Patent Term Adjustment under 37 CFR § 1.705(b) requesting reconsideration of the patent term adjustment (PTA) determination for this application for which a Notice of Allowance was mailed on June 16, 2005. Applicants request reconsideration of the 62-day reduction in PTA based on the period of January 18, 2005 through March 21, 2005. The circumstances pertaining to the time interval in question do not meet the criteria set forth under 37 CFR § 1.704(c) as an Applicant delay.

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Applicants provide herein a statement of the facts involved specifying the correct PTA and the basis under 37 CFR § 1.702 for the PTA adjustment. Applicants also note that a terminal disclaimer was filed in this case.

Applicants' Statement

Under 37 CFR § 1.704(c), when calculating the PTA adjustment, particular circumstances are delineated that are considered to constitute a failure of the Applicant to engage in reasonable efforts to conclude processing or examination of an application and that result in a reduction of the period of PTA adjustment set forth in 37 CFR § 1.703. The specific circumstances, which are set out in subsections of 37 CFR § 1.704(c), include, paragraph 8, which reads:

> Submission of a supplemental reply or other paper, other than a supplemental reply or other paper expressly requested by the examiner, after a reply has been filed, in which case the period of adjustment set forth in § 1.703 shall be reduced by the number of days, if any, beginning on the day after the date the initial reply was filed and ending on the date that the supplemental reply or other such paper was filed. (emphasis added).

Applicants submit that the period of time from Applicants' filing of a Reply to a Restriction Requirement on January 18, 2005 through Applicants' filing of an Amendment on March 17, 2005 does not meet the criteria set forth under 37 CFR §1.704(c) as a circumstance that constitutes a failure on the part of Applicants to engage in reasonable efforts to conclude processing or examination of an application. 37 CFR §1.704(c)(8) indicates that the submission of a supplemental reply or other paper, other than a supplemental reply or other paper expressly requested by the examiner after a reply has been filed is the circumstance under which reduction of PTA is warranted. In the instant case, the Amendment filed by Applicants on March 17, 2005 was expressly requested by the Examiner.

As indicated in the Interview Summary included in the Amendment filed on March 17, 2005, the Amendment was prepared and filed in response to a request by the Examiner, and it made the amendments the Examiner requested:

> Applicants present this amendment in response to the Examiner initiated telephone interview with Applicants' representatives Michael Siekman and Marie Aucoin. Applicants have amended the specification as requested to renumber the Tables consecutively as they appear in the text. Applicants have amended the Tables to reflect the amended Table numbers. Applicants have inserted Table 220 (now

> Table 21) and Table 221 (now Table 22) from U.S. Patent No. 5,663,143 which is incorporated by reference. Support for this insertion is found on page 1, lines 21-22 and page 21, lines 11-14. Applicants have added the sequence identifiers for each of the sequences in Table 5 (formerly Table 13) and corrected the sequence identifiers for the sequences in Tables 12-13 (formerly Tables 207-208) (emphasis added).

[Amendment of March 17, 2005 at p. 98 (attached)(emphasis added).] Indeed, the Examiner recorded the substance of the interview on an Examiner-Initiated Interview Summary Form, referring to the "telephonic interview initiated by the Examiner." [Examiner-Initiated Interview Summary at p. 2 (attached).] In view of the fact that the Supplemental Reply filed on March 17, 2005 was filed at the express request of the Examiner, the PTA should not have been reduced by the 62 days for the filing of the Amendment.

Applicants submit herewith the fee of $200.00 for filing an application for patent term adjustment as set forth in 37 CFR §1.18(e). If there is an additional fee occasioned by this application and request that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully Submitted,
Ley et al., Applicants

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Docket No.: D0617.70005US01
Date: September 16, 2005
x09/16/05x

DOCKET NO.: D0617.70005US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Arthur C. Ley et al.
Serial No.:	10/038,722
Confirmation No.:	4070
Filed:	January 8, 2002
For:	ITI-D1 KUNITZ DOMAIN MUTANTS AS hNE INHIBITORS
Examiner:	William W. Moore
Art Unit:	1652

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

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Michael T. Siekman, Reg. No. 36,276

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Sir:

In response to the Examiner Initiated Telephone Interview on February 10, 2005, please amend the above-identified application as follows:

Amendments to the Specification begin on page 2 of this amendment.

Remarks begin on page 98 of this amendment.

In the Specification

Please delete the following three paragraphs beginning on page 1, line 4:

"This application is a continuation of 08/849,406 filed July 21, 1999, now pending, which is a national stage of PCT/US95/16349 filed December 15, 1995, which is a continuation-in-part of application 08/358,160 filed December 16, 1994, now patented (USP 5,663,143), which is a continuation-in-part of application 08/133,031 filed February 28, 1992, now abandoned, which is the national stage of PCT/US92/01501, filed February 28, 1992.

While PCT/US92/01501 was filed as a continuation-in-part of Ladner, Guterman, Roberts, Markland, Ley, and Kent, Serial No. 07/664,989, now patented (USP 5,223,409), which is a continuation-in-part of Ladner, Guterman, Roberts, and Markland, Ser. No. 07/487,063, filed March 2, 1990, now abandoned, which is a continuation-in-part of Ladner and Guterman, Ser. No. 07/240,160, filed Sept. 2, 1988, now abandoned, the instant application does not claim §120 benefit prior to PCT/US92/01501.

All of the foregoing appications, whether or not §120 benefit is claimed, are hereby incorporated by reference."

Please add the following two new paragraphs after the Title beginning on page 1, line 4:

This application is a continuation of application serial number 08/849,406, filed July 21, 1999, now abandoned, which is a National Stage of International Application Number PCT/US95/16349, filed December 15, 1995, which is a continuation-in-part of Issued U.S. Patent Number 5,663,143, filed December 16, 1994, which is a continuation-in-part of application serial number 08/133,031, filed February 28, 1992 (abandoned), the entire disclosures of which are incorporated herein by reference.

The following applications are incorporated herein by reference. Application serial number 08/133,031, filed February 28, 1992 (abandoned), which is a National Stage of International application number PCT/US92/01501, filed February 28, 1992, which is a divisional of Issued U.S. Patent No. 5,223,409, filed March 1, 1991, which is a continuation-in-part of application serial number 07/240,160, filed September 2, 1988 (abandoned).

Please replace the paragraph beginning on page 4, line 22 with the following amended paragraph:

"Kunitz" Domain Proteinase Inhibitors. Bovine pancreatic trypsin inhibitor (BPTI, a.k.a. aprotonin) is a 58 a.a. serine proteinase inhibitor of the BPTI (Kunitz) domain (KuDom) family. Under the tradename TRASYLOL, it is used for countering the effects of trypsin released during pancreatitis. Not only is its 58 amino acid sequence known, the 3D structure of BPTI has been determined at high resolution by X-ray diffraction (HUBE77, MARQ83, WLOD84, WLOD87a, WLOD87b), neutron diffraction (WLOD84), and by NMR (WAGN87). One of the X-ray structures is deposited in the Brookhaven Protein Data Bank as "6PTI" [sic]. The 3D structure of various BPTI homologues (EIGE90, HYNE90) are also known. At least sixty homologues have been reported; the sequences of 39 homologues are given in Table ~~13~~ 5~~, and the amino acid types appearing at each position are compiled in Table 15~~. The known human homologues include domains of Lipoprotein Associated Coagulation Inhibitor (LACI) (WUNT88, GIRA89), Inter-α-Trypsin Inhibitor (ALBR83a, ALBR83b, DIAR90, ENGH89, TRIB86, GEBH86, GEBH90, KAUM86, ODOM90, SALI90), and the Alzheimer beta-Amyloid Precursor Protein. Circularized BPTI and circularly permuted BPTI have binding properties similar to BPTI (GOLD83). Some proteins homologous to BPTI have more or fewer residues at either terminus.

Please replace the paragraph beginning on page 5, line 8 with the following amended paragraph:

In BPTI, the P1 residue is at position 15. Tschesche *et al.* (TSCH87) reported on the binding of several BPTI P1 derivatives to various proteases:

Table 1

Dissociation constants for BPTI P1 derivatives, Molar.

Residue #15 P1	Trypsin (bovine pancreas)	Chymotrypsin (bovine pancreas)	Elastase (porcine pancreas)	Elastase (human leukocytes)
lysine	$6.0 \cdot 10^{-14}$	$9.0 \cdot 10^{-9}$	-	$3.5 \cdot 10^{-6}$(WT)
glycine	-	-	+	$7.0 \cdot 10^{-9}$
alanine	+	-	$2.8 \cdot 10^{-8}$	$2.5 \cdot 10^{-9}$
valine	-	-	$5.7 \cdot 10^{-8}$	$1.1 \cdot 10^{-10}$
leucine	-	-	$1.9 \cdot 10^{-8}$	$2.9 \cdot 10^{-9}$

Please replace the paragraph beginning on page 5, line 35 with the following amended paragraph:

Many mammalian species have a protein in their plasma that can be identified, by sequence homology and similarity of physical and chemical properties, as inter-α-trypsin inhibitor (ITI), a large (M_r *ca* 240,000) circulating protease inhibitor (for recent reviews see ODOM90, SALI90, GEBH90, GEBH86). The sequence of human ITI is shown in Table ~~400~~ 28. The intact inhibitor is a glycoprotein and is currently believed to consist of three glycosylated subunits that interact through a strong glycosaminoglycan linkage (ODOM90, SALI90, ENGH89, SELL87). The anti-trypsin activity of ITI is located on the smallest subunit (ITI light chain, unglycosylated M_r *ca* 15,000) which is identical in amino acid sequence to an acid stable inhibitor found in urine (UTI) and serum (STI) (GEBH86, GEBH90). The amino-acid sequence of the ITI light chain is shown in Table ~~400~~ 28. The mature light chain consists of a 21 residue N-terminal sequence, glycosylated at Ser_{10}, followed by two tandem Kunitz-type domains the first of which is glycosylated at Asn_{45} (ODOM90). In the human protein, the second Kunitz-type domain has been shown to inhibit trypsin, chymotrypsin, and plasmin (ALBR83a, ALBR83b, SELL87, SWAI88). The first domain lacks these activities but has been reported to inhibit leukocyte elastase ($\approx$1 μM>K_i>$\approx$1 nM) (ALBR83a,b, ODOM90). cDNA encoding the ITI light chain also codes for α-1-microglobulin (TRAB86, KAUM86, DIAR90); the proteins are separated post-translationally by proteolysis.

Please replace the paragraph beginning on page 10, line 16 with the following

amended paragraph:

The invention is presented as a series of examples that describe design, production, and testing of actual inhibitors and additional examples describing how other inhibitors could be discovered. The invention relates to proteins that inhibit human neutrophil elastase (hNE) with high affinity.

Table 2

NOMENCLATURE and ABBREVIATIONS

Term	Meaning
x::y	Fusion of gene *x* to gene *y* in frame.
X::Y	Fusion protein expressed from *x::y* fusion gene.
μM	Micromolar, 10^{-6} molar..
nM	Namomolar, 10^{-9} molar.
pM	Picomolar, 10^{-12} molar.

Single-letter amino-acid codes:

A: Ala	C: Cys	D: Asp	E: Glu
F: Phe	G: Gly	H: His	I: Ile
K: Lys	L: Leu	M: Met	N: Asn
P: Pro	Q: Gln	R: Arg	S: Ser
T: Thr	V: Val	W: Trp	Y: Tyr

Please replace the paragraph beginning on page 11, line 22 with the following amended paragraph:

_____There are many homologues of aprotonin, which differ from it at one or more positions but retain the fundamental structure defined above. For a given list of homologues, it is possible to tabulate the frequency of occurrence of each amino acid at each ambiguous position. (The sequence having the most prevalent amino acid at each ambiguous position is listed as "Consensus Kunitz Domain" in Table ~~100~~ 10).

Please replace the paragraph beginning on page 11, line 37 with the following

amended paragraph:

"Weak", "Moderate", "Strong" and "Very Strong" binding to and inhibition of hNE are defined in accordance with Table ~~55~~ 8. Preferably, the proteins of the present invention have a Ki of less than 1000 pM (i.e., are "strong" inhibitors), more preferably less than 50 pM, most preferably less than 10 pM (i.e., are "very strong" inhibitors).

Please replace the paragraph beginning on page 12, line 5 with the following amended paragraph:

For purposes of the present invention, an aprotonin-like Kunitz domain may be divided into ten segments, based on the consensus sequence and the location of the catalytic site. Using the amino acid numbering scheme of aprotonin, these segments are as follows (see Table ~~100~~ 10):

1: 1-4 (residues before first Cys)
2: 5-9 (first Cys and subsequent residues before P6)
3: 10-13 (P6 to P3)
4: 14 (second Cys; P2)
5: 15-21 (P1, and P1' to P6')
6: 22-30 (after P6 and up to and incl. third Cys.)
7: 31-36 (after third Cys and up to consensus Gly-Cys)
8: 37-38 (consensus Gly-Cys)
9: 39-42 (residues after Gly-Cys and before consensus [Asn|Gly]
10: 43-55 (up to last Cys)(also includes residues after last Cys, if any)

Please replace the paragraph beginning on page 13, line 24 with the following amended paragraph:

Proteins of the present invention include those comprising a Kunitz domain that is substantially homologous to the reference proteins EPI-HNE-3, EPI-HNE-4, DPI.1.1, DPI.1.2, DPI.1.3, DPI.2.1, DPI.2.2, DPI.2.3, DPI.3.1, DPI.3.2, DPI.3.3, DPI.4.1, DPI.4.2, DPI.4.3, DPI.5.1, DPI.5.2, DPI.5.3, DPI.6.1, DPI.6.2, DPI.6.3, DPI.6.4, DPI.6.5, DPI.6.6, DPI.6.7, DPI.7.1, DPI.7.2, DPI.7.3, DPI.7.4, DPI.7.5, DPI.8.1, DPI.8.2, DPI.8.3, DPI.9.1, DPI.9.2, or DPI.9.3, as defined in Table ~~100~~ 10. Homologues of EPI-HNE-3 and EPI-HNE-4 are especially preferred.

Please replace the paragraph beginning on page 15, line 14 with the following amended paragraph:

Preferred proteins of the present invention are further characterized by one of more of the preferred, highly preferred, or most preferred mutations set forth in Table ~~711~~ 41.

Please replace the paragraph beginning on page 15, line 22 with the following amended paragraph:

Claim 1 of PCT/US92/01501 refers to proteins denoted EpiNEalpha, EpiNE1, EpiNE2, EpiNE3, EpiNE4, EpiNE5, EpiNE6, EpiNE7, and EpiNE8. Claim 3 refers to proteins denoted ITI-E7, BITI-E7, BITI-E&-1222, AMINO1, AMINO2, MUTP1, BITI-E7-141, MUTT26A, MUTQE, and MUT1619. (With the exception of EpiNEalpha, the sequences of all of these domains appears in Table ~~100.~~10). Claims 4-6 related to inhibitors which are homologous to, but not identical with, the aforementioned inhibitors. These homologous inhibitors could differ from the lead inhibitors by one or more class A substitutions (claim 4), one or more class A or B substitutions (claim 5), or one or more class A, B or C substitutions (claim 6). Class A, B and C substitutions were defined in Table 65 of PCT/US92/01501. For convenience, Table 65 has been duplicated in this specification (Table 9).

Please replace the paragraph beginning on page 19, line 14 with the following amended paragraph:

Based on these data and excluding the six cysteines, we judge that the KuDom structure will allow those substitutions shown in Table ~~65~~ 9. The class indicates whether the substitutions: A) are very likely to give a stable protein having substantially the same binding to hNE, hCG, or some other serine protease as the parental sequence, B) are likely to give similar binding as the parent, or C) are likely to give a proteins retaining the KuDom structure, but which are likely to affect the binding. Mutants in class C must be tested for affinity, which is relatively easy using a display-phage system, such as the one set forth in W0/02809. The affinity of hNE and hCG inhibitors is most sensitive to substitutions at positions 15, 16, 17, 18, 34, 39, 19, 13, 11, 20, 36 of BPTI, if the inhibitor is a mutant of ITI-D1, these positions must be converted to their ITI-D1 equivalents by

aligning the cysteines in BPTI and ITI-D1.

Please replace the two paragraphs beginning on page 20, line 28 with the two following amended paragraphs:

Tables ~~207~~ 12 and ~~208~~ 13 present the sequences of additional novel BPTI mutants with high affinity for hNE. We believe these mutants to have an affinity for hNE which is about an order of magnitude higher than that of BPTI (K15V, R17L). All of these mutants contain, besides the active site mutations shown in the Tables, the MGNG mutation at positions 39-42.

Although BPTI has been used in humans with very few adverse effects, a KuDom having much higher similarity to a human KuDom poses much less risk of causing an immune response. Thus, we transferred the active site changes found in EpiNE7 into the first KuDom of inter-α-trypsin inhibitor. For the purpose of this application, the numbering of the nucleic acid sequence for the ITI light chain gene is that of TRAB86 and that of the amino acid sequence is the one shown for UTI in FIg. 1 of GEBH86. The necessary coding sequence for ITI-DI is the 168 bases between positions 750 and 917 in the cDNA sequence presented in TRAB86. The amino acid sequence of human ITI-D1 is 56 amino acids long, extending from Lys-22 to Arg-77 of the complete ITI light chain sequence. The P1 site of ITI-DI is Met-36. Tables ~~220-221~~ 21-22 present certain ITI mutants; note that the residues are numbered according to the homologus Kunitz domain of BPTI, i.e., with the P1 residue numbered 15. It should be noted that it is probably acceptable to truncate the amino-terminal of ITI-D1, at least up to the first residue homologous with BPTI.

Please replace the paragraph beginning on page 21, line 35 with the following amended paragraph:

In a second series of embodiments, the present invention relates to Kunitz-type domains which inhibit HNE, but excludes those domains corresponding exactly to the lead domains of claims 1 and 3 of PCT/US92/01501. Preferably, such domains also differ from these lead domains by one or more mutations which are not class A substitutions, more preferably, not class A or B substitutions, and still more preferably, not class A, B or C substitutions, as defined in Table ~~65~~ 9. Desirably, such domains are each more similar

to one of the aforementioned reference proteins than to any of the lead proteins set forth in PCT/US92/01501.

Please replace the paragraph beginning on page 23, line 1 with the following amended paragraph:

Example 1: Expression and display of BPTI, ITI-D1, and other Kunitz Domains.

Table ~~30~~ 6 shows a display gene that encodes: 1) the M13 III signal peptide, 2) BPTI, and 3) the first few amino-acids of mature M13 III protein. Phage have been made in which this gene is the only *iii*-like gene so that all copies of III expressed are expected to be modified at the amino terminus of the mature protein. Substitutions in the BPTI domain can be made in the cassettes delimited by the *Acc*III, *Xho*I, *Pfl*MI, *Apa*I, *Bss*HII, *Stu*I, *Xca*I, *Esp*I, *Sph*I, or *Nar*I (same recognition as *Kas*I) sites. Table ~~100~~ 10 gives amino-acid sequences of a number of Kunitz domains, some of which inhibit hNE. Each of the hNE-inhibiting sequences shown in Table ~~100~~ 10 can be expressed as an intact hNE-binding protein or can be incorporated into a larger protein as a domain. Proteins that comprise a substantial part of one of the hNE-inhibiting sequences found in Table ~~100~~ 10 are expected to exhibit hNE-inhibitory activity. This is particularly true if the sequence beginning with the first cysteine and continuing through the last cysteine is retained.

Please replace the paragraph beginning on page 23, line 31 with the following amended paragraph:

Table ~~35~~ 7 gives the sequence of a fusion gene comprising: a) the signal sequence of M13 III, b) ITI-D1, and c) the initial part of mature III of M13. The displayed ITI-D1 domain can be altered by standard methods including: i) oligonucleotide-directed mutagenesis of single-stranded phage DNA, and ii) cassette mutagenesis of RF DNA using the restriction sites (*Bgl*I, *Eag*I, *Nco*I, *Sty*I, *Pst*I, and *Kas*I (two sites)) designed into the gene.

Please replace the paragraph beginning on page 24, line 14 with the following amended paragraph:

The results of several fractionations are shown in Table ~~212~~ 14 (EpiNE-7 or MA-ITI-D1 phage bound to hNE beads). The pH elution profiles obtained using the control

display phage (EpiNE-7) were similar previous profiles (US 5,223,409). About 0.3% of the EpiNE-7 display phage applied to the hNE beads eluted during the fractionation procedure and the elution profile had a maximum for elution at about pH 4.0.

Please replace the two paragraphs beginning on page 25, line 5 with the two following amended paragraphs:

Example 3: Alteration of the P1 region of ITI-D1.

We assume that ITI-D1 and EpiNE-7 have the same 3D configuration in solution as BPTI. Although EpiNE-7 and ITI-D1 are identical at positions 13, 17, 20, 32, and 39, they differ greatly in their affinities for hNE. To improve the affinity of ITI-D1 for hNE, the EpiNE-7 sequence **Val**$_{15}$-**Ala**$_{16}$-Met$_{17}$-**Phe**$_{18}$-**Pro**$_{19}$-Arg$_{20}$ SEQ ID NO:130 (**bold**, underscored amino acids are alterations) was incorporated into the ITI-D1 sequence by cassette mutagenesis between the *Eag*I and *StyI*/*Nco*I sites shown in Table ~~35~~ 7. Phage isolates containing the ITI-D1::III fusion gene with the EpiNE-7 changes around the P1 position are called MA-ITI-D1E7.

Example 4: Fractionation of MA-ITI-D1E7 phage.

To test if ITI-D1E7-display phage bind hNE beads, pH elution profiles were measured. Aliquots of EpiNE-7, MA-ITI-D1, and MA-ITI-D1E7 display phage were incubated with hNE beads for three hours at room temperature (RT). The beads were washed and phage were eluted as described in US 5,223,409, except that only three pH elutions were performed. These data are in Table ~~215~~ 16. The pH elution profile of EpiNE-7 display phage is as described. MA-ITI-D1E7 phage show a broad elution maximum around pH 5. The total fraction of MA-ITI-D1E7 phage obtained on pH elution from hNE beads was about 40-fold less than that obtained using EpiNE-7 display phage.

Please replace the paragraph beginning on page 27, line 33 with the following amended paragraph:

We characterized the binding properties to hNE-beads of MA-BITI and MA-BITI-E7 display phage using the extended pH fractionation procedure described in US 5,223,409. The results are in Table ~~216~~ 17. The pH elution profiles for MA-BITI and MA-BITI-E7 show significant differences from the profiles exhibited by MA-ITI-D1 and MA-ITI-

D1E7. In both cases, the alterations at the putative amino terminus of the displayed fusion protein produce a several-fold increase in the fraction of the input display phage eluted from the hNE-beads.

Please replace the paragraph beginning on page 28, line 5 with the following amended paragraph:

The binding capacity of hNE-beads for display phage varies among preparations of beads and with age for each individual preparation of beads. Thus, it is difficult to directly compare absolute yields of phage from elutions performed at different times. For example, the fraction of MA-EpiNE7 display phage recovered from hNE-beads varies two-fold among the experiments shown in Tables ~~212, 215, and 216~~ 14, 16, and 17. However, the shapes of the pH elution profiles are similar. It is possible to correct somewhat for variations in binding capacity of hNE-beads by normalizing display phage yields to the total yield of MA-EpiNE7 phage recovered from the beads in a concurrent elution. When the data shown in Tables ~~212, 215, and 216~~ 14, 16, and 17 are so normalized, the recoveries of display phage, relative to recovered MA-EpiNE7, are shown in Table ~~10~~ 3.

Table ~~10~~ 3: Recovery of Display phage.

Display Phage strain	Normalized fraction of input
MA-ITI-D1	0.0067
MA-BITI	0.018
MA-ITI-D1E7	0.027
MA-BITI-E7	0.13

Please replace the paragraph beginning on page 29, line 36 with the following amended paragraph:

ITI-D1 derivative BITI-E7-1222 is BITI-E7 with the alteration A11T. ITI-D1 derivative BITI-E7-141 is BITI-E7 with the alterations E31Q and Q34V; phage that ~~dhe~~ display the presence of ~~tisplay~~ these proteins are MA-BITI-E7-1222 and MA-BITI-E7-141. We determined the binding properties to hNE-beads of MA-BITI-E7-1222 and MA-BITI-E7-141 display phage using the extended pH fractionation protocol described

previously. The results are in Tables ~~217~~ 18 (for MA-BITI-E7 and MA-BITI-E7-1222) and ~~218~~ 19 (for MA-EpiNE7 and MA-BITI-E7-141). The pH elution profiles for the MA-BITI-E7 and MA-BITI-E7-1222 phage are almost identical. Both phage strains exhibit pH elution profiles with identical maxima (between pH 5.0 and pH 4.5) as well as the same total fraction of input phage eluted from the hNE-beads (0.03%). Thus, the T11A substitution in the displayed ITI-D1 derivative has no appreciable effect on the binding to hNE-beads.

Please replace the paragraph beginning on page 30, line 36 with the following amended paragraph:

Example 7: Mutagenesis of BITI-E7-141

BITI-E7-141 differs from ITI-D1 at nine positions (1, 2, 4, 15, 16, 18, 19, 31, and 34). To obtain the protein having the fewest changes from ITI-D1 while retaining high specific affinity for hNE, we have investigated the effects of reversing the changes at positions 1, 2, 4, 16, 19, 31, and 34. The derivatives of BITI-E7-141 that were tested are MUT1619, MUTP1, and MUTT26A. The derivatives of BITI that were tested are AMINO1 and AMINO2. The derivative of BITI-E7 that was tested is MUTQE. All of these sequences are shown in Table ~~100~~ 10. MUT1619 restores the ITI-D1 residues Ala_{16} and Ser_{19}. The sequence designated "MUTP1" asserts the amino acids I_{15}, G_{16}, S_{19} in the context of BITI-E7-141. It is likely that M_{17} and F_{18} are optimal for high affinity hNE binding. G_{16} and S_{19} occurred frequently in the high affinity hNE-binding BPTI-variants obtained from fractionation of a library of BPTI-variants against hNE (ROBE92). Three changes at the putative amino terminus of the displayed ITI-D1 domain were introduced to produce the MA-BITI series of phage. AMINO1 carries the sequence K_1-E_2 while AMINO2 carries K_1-S_4. Other amino acids in the amino-terminal region of these sequences are as in ITI-D1. MUTQE is derived from BITI-E7-141 by the alteration Q31E (reasseting the ITI-D1 w.t. residue). Finally, the mutagenic oligonucleotide MUTT26A is intended to remove a potential site of N-linked glycosylation, N_{24}-G_{25}-T_{26}. In the intact ITI molecule isolated from human serum, the light chain polypeptide is glycosylated at this site (N_{45}, ODOM90). It is likely that N_{24} will be glycosylated if the BITI-E7-141 protein is produced *via* eukaryotic expression. Such glycosylation may render the protein immunogenic when used for long-term treatment. The MUTT26A contains the alteration

T26A and removes the potential glycosylation site with minimal changes in the overall chemical properties of the residue at that position. In addition, an Ala residue is frequently found in other BPTI homologues at position 26 (see Table 34 of US 5,223,409). Mutagenesis was performed on ssDNA of MA-BITI-E7-141 phage.

Please replace the paragraph beginning on page 31, line 37 with the following amended paragraph:

Example 8: hNE-binding properties of mutagenized MA-BITI-E7-141 display phage

Table ~~219~~ 20 shows pH elution data for various display phage eluted from hNE-beads. Total pfu applied to the beads are in column two. The fractions of this input pfu recovered in each pH fraction of the abbreviated pH elution protocol (pH 7.0, pH 3.5, and pH 2.0) are in the next three columns. For data obtained using the extended pH elution protocol, the pH 3.5 listing represents the sum of the fractions of input recovered in the pH 6.0, pH 5.5, pH 5.0, pH 4.5, pH 4.0, and pH 3.5 elution samples. The pH 2.0 listing is the sum of the fractions of input obtained from the pH 3.0, pH 2.5, and pH 2.0 elution samples. The total fraction of input pfu obtained throughout the pH elution protocol is in the sixth column. The final column of the table lists the total fraction of input pfu recovered, normalized to the value obtained for MA-BITI-E7-141 phage.

Please replace the two paragraphs beginning on page 32, line 16 with the two following amended paragraphs:

Two factors must be considered when making comparisons among the data shown in Table ~~219~~ 20. The first is that due to the kinetic nature of phage release from hNE-beads and the longer time involved in the extended pH elution protocol, the fraction of input pfu recovered in the pH 3.5 fraction will be enriched at the expense of the pH 2.0 fraction in the extended protocol relative to those values obtained in the abbreviated protocol. The magnitude of this effect can be seen by comparing the results obtained when MA-BITI-E7-141 display phage were eluted from hNE-beads using the two protocols. The second factor is that, for the range of input pfu listed in Table ~~219~~ 20, the input pfu influences recovery. The greater the input pfu, the greater the total fraction of the input recovered in the elution. This effect is apparent when input pfu differ by more than a factor of about 3 to 4. The effect can lead to an overestimate of affinity of display phage for hNE-beads when data from phage applied at higher titers is compared with that from phage applied at

lower titers.

With these caveats in mind, we can interpret the data in Table ~~219~~ 20. The effects of the mutations introduced into MA-BITI-E7-141 display phage ("parental") on binding of display phage to hNE-beads can be grouped into three categories: those changes that have little or no measurable effects, those that have moderate (2- to 3-fold) effects, and those that have large (>5-fold) effects.

Please replace the paragraph beginning on page 33, line 28 with the following amended paragraph:

On the basis of the above interpretations of the data in Table ~~219~~ 20, we can conclude that:

1.) The substitution of ALA for THR at position 26 in ITI-D1 and its derivatives has no effect on the interaction of the inhibitor with hNE. Thus, the possibility of glycosylation at Asn_{24} of an inhibitor protein produced in eukaryotic cell culture can be avoided with no reduction in affinity for hNE.

2.) The increase in affinity of display phage for hNE-beads from the changes E31Q and Q34V results primarily from the Val substitution at 34.

3.) All three changes at the amino terminal region of ITI-D1 (positions 1,2, and 4) influence display phage binding to hNE-beads to varying extents. The S4F alteration seems to have about the same effect as does E2P. The change at position 1 appears to have only a small effect.

4.) The changes in the region around the P1 residue in BITI-E7-141 (position 15) influence display phage binding to hNE. The changes A16G and P19S appear to reduce the affinity of the inhibitor somewhat (perhaps 3-fold). The substitution of I15V further reduces binding.

Please replace the paragraph beginning on page 34, line 23 with the following amended paragraph:

Summary: estimated affinities of isolated ITI-D1 derivatives for hNE

On the basis of display phage binding to and elution from hNE beads, it is possible to estimate affinities for hNE that various derivatives of ITI-D1 may display free in solution. These estimates are summarized in Table ~~55~~ 8.

Please replace the paragraph beginning on page 35, line 2 with the following amended paragraph:

Example 9: Amino-acid sequences of EPI-HNE-3 and EPI-HNE-4

Table ~~100~~ 10 gives amino acid sequences of four human-neutrophil-elastase (hNE) inhibitor proteins: EPI-HNE-1 (identical to EpiNE1), EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4. These proteins have been derived from the parental Kunitz-type domains shown. Each of the proteins is shown aligned to the parental domain using the six cysteine residues (shaded) characteristic of the Kunitz-type domain. Residues within the inhibitor proteins that differ from those in the parental protein are in upper case. Entire proteins having the sequences EPI-HNE-1, EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4 (Table ~~100~~ 10) have been produced. Larger proteins that comprise one of the hNE-inhibiting sequences are expected to have potent hNE-inhibitory activity; EPI-HNE-1, EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4 are particularly preferred. It is expected that proteins that comprise a significant part of one of the hNE-inhibiting sequences found in Table ~~100~~ 10 (particularly if the sequence starting at or before the first cysteine and continuing through or beyond the last cysteine is retained) will exhibit potent hNE-inhibitory activity.

Please replace the paragraph beginning on page 35, line 32 with the following amended paragraph:

EPI-HNE-3 is derived from the second Kunitz domain of the light chain of the human inter-α-trypsin inhibitor protein (ITI-D2). The amino acid sequence of EPI-HNE-3 differs from that of ITI-D2(3-58) at only four positions: R15I, I18F, Q19P and L20R. EPI-HNE-4 differs from EPI-HNE-3 by the substitution A3E (the amino-terminal residue) which both facilitates secretion of the protein in *P. pastoris* and improves the K_D for hNE. Table ~~602~~ 30 gives some physical properties of the hNE inhibitor proteins. All four proteins are small, high-affinity (K_i=2 to 6 pM), fast-acting (k_{on}=4 to 11 x10^6 $M^{-1}s^{-1}$) inhibitors of hNE.

Please replace the two paragraphs beginning on page 36, line 11 with the two following amended paragraphs:

Example 10: *Pichia pastoris* production system.

Transformed strains of *Pichia pastoris* were used to express the various EPI-HNE proteins derived from BPTI and ITI-D2. Protein expression cassettes are cloned into the plasmid pHIL-D2 using the *Bst*BI and *Eco*RI sites (Table ~~111~~ 11). The DNA sequence of pHIL-D2 is given in Table ~~250~~ 23. The cloned gene is under transcriptional control of *P. pastoris* upstream (labeled "aox1 5'") *aox1* gene promoter and regulatory sequences (dark shaded region) and downstream polyadenylation and transcription termination sequences (second cross-hatched region, labeled "aox1 3'"). *P. pastoris* GS115 is a mutant strain containing a non-functional histidinol dehydrogenase (*his4*) gene. The *his4* gene contained on plasmid pHIL-D2 and its derivatives can be used to complement the histidine deficiency in the host strain. Linearization of plasmid pHIL-D2 at the indicated *Sac*I site directs plasmid incorporation into the host genome at the *aox1* locus by homologous recombination during transformation. Strains of *P. pastoris* containing integrated copies of the expression plasmid will express protein genes under control of the *aox1* promoter when the promoter is activated by growth in the presence of methanol as the sole carbon source.

We have used this high density *Pichia pastoris* production system to produce proteins by secretion into the cell CM. Expression plasmids were constructed by ligating synthetic DNA sequences encoding the *S. cerevisiae* mating factor α prepro peptide fused directly to the amino terminus of the desired hNE inhibitor into the plasmid pHIL-D2, using the *Bst*BI and the *Eco*RI sites shown. Table ~~251~~ 24 gives the DNA sequence of a *Bst*BI-to-*Eco*RI insert that converts pHIL-D2 into pHIL-D2(MFα-PrePro::EPI-HNE-3). In this construction, the fusion protein is placed under control of the upstream inducible *P. pastoris aox1* gene promoter and the downstream *aox1* gene transcription termination and polyadenylation sequences. Expression plasmids were linearized by *Sac*I digestion and the linear DNA was incorporated by homologous recombination into the genome of the *P. pastoris* strain GS115 by spheroplast transformation. Regenerated spheroplasts were selected for growth in the absence of added histidine, replated, and individual isolates were screened for methanol utilization phenotype (*mut*$^+$), secretion levels, and gene dose (estimated via Southern hybridization experiments). High level secretion stains were selected for production of hNE inhibitors: PEY-33 for production of EPI-HNE-2 and PEY-43 for production of EPI-HNE-3. In both of these strains, we estimate that four copies of the expression plasmid are integrated as a tandem array into the *aox1* gene locus.

Please replace the paragraphs beginning on page 37, line 20 with the following amended paragraph:

To facilitate alteration of the Kunitz-domain encoding segment of pHIL-D2 derived plasmids, we removed two restriction sites given in Table ~~111~~ 11: the *Bst*BI at 4780 and the *Aat*II site at 5498. Thus, the Kunitz-domain encoding segment is bounded by unique *Aat*II and *Eco*RI sites. The new plasmids are called pD2pick("insert") where "insert" defines the domain encoded under control of the *aox1* promoter. Table ~~253~~ 26 gives the DNA sequence of pD2pick(MFα::EPI-HNE-3). Table ~~254~~ 27 gives a list of restriction sites in pD2pick(MFα::EPI-HNE-3).

EPI-HNE-4 is encoded by pD2pick(MFαPrePro::EPI-HNE-4) which differs from pHIL-D2 in that: 1) the *Aat*II/*Eco*RI segment of the sequence given in Table ~~251~~ 24 is replaced by the segment shown in Table ~~252~~ 25 and 2) the changes in the restriction sites discussed above have been made. Strain PEY-53 is *P. pastoris* GS115 transformed with pD2pick(MFα::EPI-HNE-4).

Please replace the paragraph beginning on page 38, line 21 with the following amended paragraph:

Table ~~607~~ 34 and Table ~~608~~ 35 give the kinetics of cell growth (estimated as A_{600}) and protein secretion (mg/l) for cultures of PEY-33 and PEY-43 during the methanol-limited feed portions of the relevant fermentations. Concentrations of the inhibitor proteins in the fermentation cultures were determined from *in vitro* assays of hNE inhibition by diluted aliquots of cell-free culture media obtained at the times indicated. Despite similarities in gene dose, fermentation conditions, cell densities, and secretion kinetics, the final concentrations of inhibitor proteins secreted by the two strains differ by nearly an order of magnitude. The final concentration of EPI-HNE-2 in the PEY-33 fermentation CM was 720 mg/l. The final concentration of EPI-HNE-3 in the PEY-43 fermentation CM was 85 mg/l. The differences in final secreted protein concentrations may result from idiosyncratic differences in the efficiencies with which the yeast synthesis and processing systems interact with the different protein sequences.

Please replace the paragraph beginning on page 39, line 1 with the following

amended paragraph:

Strain PEY-33 secreted EPI-HNE-2 protein into the CM as a single molecular species which amino acid composition and N-terminal sequencing reveled to be the correctly-processed Kunitz domain with the sequence shown in Table ~~601~~ 29. The major molecular species produced by PEY-43 cultures was the properly-processed EPI-HNE-3 protein. However, this strain also secreted a small amount (about 15% to 20% of the total EPI-HNE-3) of incorrectly-processed material. This material proved to be a mixture of proteins with amino terminal extensions (primarily nine or seven residues in length) arising from incorrect cleavage of the MF α PrePro leader peptide from the mature Kunitz domain. The correctly processed protein was purified substantially free of these contaminants as described below.

Please replace the paragraph beginning on page 39, line 24 with the following amended paragraph:

Example 12: Purification of EPI-HNE-2.

Table ~~603~~ 31 gives particulars of the purification of EPI-HNE-2, lot 1. The PEY-33 fermenter culture was harvested by centrifugation at 8000 x g for 15 min and the cell pellet was discarded. The 3.3 liter supernatant fraction was microfiltered used a Minitan Ultrafiltration System (Millipore Corporation, Bedford, MA) equipped with four 0.2μ filter packets.

Please replace the paragraphs beginning on page 41, line 7 with the following amended paragraph:

Table ~~603~~ 31 summarizes the yields and relative purity of EPI-HNE-2 at various steps in the purification procedure. The overall yield of the purification procedure was about 30%. The major source of loss was retention of material in the retentate fractions of the 0.2μ microfiltration and 30k ultrafiltration steps.

Example 13: Purification of EPI-HNE-3.

Purification of EPI-HNE-3, lot 1, is set out in Table ~~604~~ 32. The PEY-43 fermenter culture was harvested by centrifugation at 8,000 x g for 15 min and the cell pellet was discarded. The supernatant solution (3100 ml) was microfiltered through 0.2μ Minitan

packets (4 packets). After the concentration, a diafiltration of the retentate was performed so that the final filtrate volume from the 0.2μ filtration was 3300 ml.

Please replace the paragraph beginning on page 43, line 17 with the following amended paragraph:

Table ~~604~~ 32 gives the yield and relative purity of EPI-HNE-3 at various steps in the purification procedure. A major purification step occurred at the first ion exchange chromatography procedure. The ammonium sulfate precipitation step provided only a small degree of further purification. Some loss of inhibitor activity occurred on incubation at pH=9 (See pH stability data). The production and purification of EPI-HNE-1 and EPI-HNE-4 were analogous to that of EPI-HNE-2 and EPI-HNE-3.

Please replace the paragraph beginning on page 45, line 14 with the following amended paragraph:

We recorded data used to determine K_i for EPI-HNE-2 and EPI-HNE-3 reacting with hNE. Data obtained as described above are recorded as percent residual activity plotted as a function of added inhibitor. Values for K_i and for active inhibitor concentration in the stock are obtained from a least-squares fit program. From the data, K_i values for EPI-HNE-2 and for EPI-HNE-3 reacting with hNE at RT were calculated to be 4.8 pM and 6.2 pM, respectively. Determinations of K_i for EPI-HNE-2 and EPI-HNE-3 reacting with hNE are given in Table ~~610~~ 36 and Table ~~611~~ 37.

Please replace the five paragraphs beginning on page 46, line 8 with the five following amended paragraphs:

The kinetic off rate, k_{off}, is calculated from the measured values of K_i and k_{on} as:

$$k_{off} = K_D \times k_{on}$$

The values from such measurements are included in Table ~~602~~ 30. The EPI-HNE proteins are small, high affinity, fast acting inhibitors of hNE.

B. Specificity.

Example 16: Specificity of EPI-HNE proteins

We attempted to determine inhibition constants for EPI-HNE proteins reacting with

several serine proteases. The results are summarized in Table ~~605~~ 33. In all cases except chymotrypsin, we were unable to observe any inhibition even when 10 to 100 μM inhibitor was added to enzyme at concentrations in the nM range. In Table ~~605~~ 33, our calculated values for K_i (for the enzymes other than chymotrypsin) are based on the conservative assumption of less than 10% inhibition at the highest concentrations of inhibitor tested. For chymotrypsin, the K_i is about 10 μM and is probably not specific.

C. In Vitro Stability.

Example 17: Resistance to Oxidative Inactivation.

Table ~~620~~ 39 shows measurements of the susceptibility of EPI-HNE proteins to oxidative inactivation as compared with that of two other natural protein hNE inhibitors: α 1 Protease Inhibitor (API) and Secretory Leucocyte Protease Inhibitor (SLPI). API (10 μM), SLPI (8.5 μM), EPI-HNE-1 (5 μM), EPI-HNE-2 (10 μM), EPI-HNE-3 (10 μM); and EPI-HNE-4 (10 μM) were exposed to the potent oxidizing agent, Chloramine-T, at the indicated oxidant:inhibitor ratios in 50 mM phosphate buffer, pH=7.0 for 20 minutes at RT. At the end of the incubation period, the oxidation reactions were quenched by adding methionine to a final concentration of 4 mM. After a further 10 minute incubation, the quenched reactions were diluted and assayed for residual inhibitor activity in our standard hNE-inhibition assay.

Both API and SLPI are inactivated by low molar ratios of oxidant to inhibitor. The Chloramine-T:protein molar ratios required for 50% inhibition of API and SLPI are about 1:1 and 2:1, respectively. These ratios correspond well with the reported presence of two and four readily oxidized methionine residues in API and SLPI, respectively. In contrast, all four EPI-HNE proteins retain essentially complete hNE-inhibition activity following exposure to Chloramine-T at all molar ratios tested (up to 50:1, in the cases of EPI-HNE-3 and EPI-HNE-4). Neither EPI-HNE-3 nor EPI-HNE-4 contain any methionine residues. In contrast, EPI-HNE-1 and EPI-HNE-2 each contains two methionine residues (see Table ~~100~~ 10). The resistance of these proteins to oxidative inactivation indicates that the methionine residues are either inaccessible to the oxidant or are located in a region of the protein that does not interact with hNE.

Example 18: pH Stability.

Table ~~612~~ 38 shows the results of measurements of the pH stability of EPI-HNE proteins. The stability of the proteins to exposure to pH conditions in the range of pH 1 to pH 10 was assessed by maintaining the inhibitors in buffers of defined pH at 37°C for 18 hours and determining the residual hNE inhibitory activity in the standard hNE-inhibition assay. Proteins were incubated at a concentration of 1 μM. The buffers shown in Table ~~14~~ 4 were formulated as described (STOL90) and used in the pH ranges indicated:

Table ~~14~~ 4: Buffers used in stability studies

Buffer	Lowest pH	Highest pH
Glycine-HCl	1	2.99
Citrate-Phosphate	3	7
Phosphate	7	8
Glycine-NaOH	8.5	10

Please replace the paragraph beginning on page 48, line 22 with the following amended paragraph:

Example 19: Temperature Stability.

The stability of EPI-HNE proteins to temperatures in the range 0°C to 95°C was assessed by incubating the inhibitors for thirty minutes at various temperatures and determining residual inhibitory activity for hNE. In these experiments, protein concentrations were 1 μM in phosphate buffer at pH=7. As is shown in Table ~~630~~ 40, the four inhibitors are quite temperature stable.

Please replace the two paragraphs beginning on page 49, line 16 with the two following amended paragraphs:

Example 21: Substitution of Segments in Kunitz Domains

Table ~~100~~ 10 shows the amino-acid sequences of 11 human Kunitz domains. These sequences have been broken into ten segments: 1:N terminus-residue 4; 2:residue 5; 3:6-9(or 9a); 4:10-13; 5:14; 6:15-21; 7:22-30, 8:31-36; 8:37-38; 9:39-42; and 10:43-C terminus (or 42a-C terminus).

Segments 1, 3, 5, 7, and 9 contain residues that strongly influence the binding properties of Kunitz domains and are double underscored in the Consensus Kunitz Domain of Table ~~100~~ 10. Other than segment 1, all the segments are the same length except for TFPI-2 Domain 2 which carries an extra residue in segment 2 and two extra

residues in segment 10.

Please replace the paragraph beginning on page 50, line 2 with the following amended paragraph:

It may be desirable to have an hNE inhibitor that is highly similar to a human protein to reduce the chance of immunogenicity. Candidate high-affinity hNE inhibitor protein sequences may be obtained by taking an aprotonin-type Kunitz domain that strongly or very strongly inhibits hNE, and replacing one, two, three, four or all of segments 2, 4, 6, 8, and 10 with the corresponding segment from a human Kunitz domain, such as those listed in Table ~~100~~ 10, or other domain known to have relatively low immunogenicity in humans. (Each of segments 2, 4, 6, 8, and 10 may be taken from the same human domain, or they may be taken from different human domains.) Alternatively, a reduced immunogenicity, high hNE inhibiting domain may be obtained by taking one of the human aprotonin-type Kunitz domains and replacing one, two, three or all of segments 3, 5, 7 and 9 (and preferably also segment 1) with the corresponding segment from one or more aprotonin-like Kunitz domains that strongly or very strongly inhibit hNE. In making these humanized hNE inhibitors, one may, of course, use, rather than a segment identical to that of one of the aforementioned source proteins, a segment which differs from the native source segment by one or more conservative modifications. Such differences should, of course, be taken with due consideration for their possible effect on inhibitory activity and/or immunogenicity. In some cases, it may be advantageous that the segment be a hybrid of corresponding segments from two or more human domains (in the case of segments 2, 4, 6, 8 and 10) or from two or more strong or very strong hNE inhibitor domains (in the case of segments 3, 5, 7, and 9). Segment 1 may correspond to the segment 1 of a strong or very strong hNE inhibitor, or the segment 1 of a human aprotonin-like Kunitz domain, or be a chimera of segment 1's from both.

Please replace the paragraph beginning on page 51, line 27 with the following amended paragraph:

All of the protein sequences mentioned in this example are to be found in Table ~~100~~ 10. Designed protease inhibitors are designated "DPI" and are derived from human Kunitz domains (also listed in Table ~~100~~ 10). Each of the sequences designated DPI.i.2

(for i = 1 to 9) is derived from the domain two above it in the table by making minimal point mutations. Each of the sequences designated DPI.i.3 (for i = 1 to 9) is derived from the sequence three above it by more extensive mutations intended to increase affinity. For some parental domains, additional examples are given. The sequences designated DPI.i.1 are discussed in Example 21.

Please replace the paragraph beginning on page 52, line 8 with the following amended paragraph:

The Kunitz domains having very high affinity for hNE herein disclosed (as listed in Table ~~100~~ 10) have no charged groups at residues 10, 12 through 19, 21, and 32 through 42. At position 11, only neutral and positively charged groups have been observed in very high affinity hNE inhibitors. At position 31, only neutral and negatively charged groups have been observed in high-affinity hNE inhibitors. If a parental Kunitz domain has a charged group at any of those positions where only neutral groups have been observed, then each of the charged groups is preferably changed to an uncharged group picked from the possibilities in Table ~~790~~ 46 as the next step in improving binding to hNE. Similarly, negatively charged groups at 11 and 19 and positively charged groups at 31 are preferably replaced by groups picked from Table ~~790~~ 46.

Please replace the paragraph beginning on page 54, line 11 with the following amended paragraph:

The above mutations are summarized in Table ~~711~~ 41. Table ~~711~~ 41 contains, for example, mutations of the form X15I which means change the residue at position 15 (whatever it is) to Ile or leave it alone if it is already Ile. A Kunitz domain that contains the mutation X18F and either X15I or X15V (X15I preferred) will have strong affinity for hNE. As from one up to about 8 of the mutations found in Table ~~711~~ 41 are asserted, the affinity of the protein for hNE will increase so that the K_i approaches the range 1-5 pM.

Please replace the paragraphs beginning on page 56, line 7 with the following amended paragraph:

Example 23: Libraries of Kunitz Domains

Other Kunitz domains that can potently inhibit hNE may be derived from human Kunitz

domains either by substituting hNE-inhibiting sequences into human domains or by using the methods of US 5,223,409 and related patents. Table ~~720~~ 42 shows a gene that will cause display of human LACI-D2 on M13 gIIIp; essentially the same gene could be used to achieve display on M13 gVIIIp or other anchor proteins (such as bacterial outer-surface proteins (OSPs)). Table ~~725~~ 43 shows a gene to cause display of human LACI D1.

Table ~~730~~ 44 and Table ~~735~~ 45 give variegations of LACI-D1 and LACI-D2 respectively. Each of these is divided into variegation of residues 10-21 in one segment and residues 31-42 in another. In each case, the appropriate vgDNA is introduced into a vector that displays the parental protein and the library of display phage are fractionated for binding to immobilized hNE.

Please replace Table 13 beginning on page 57 to page 66 with the following amended Table:

Table ~~13~~ 5: BPTI Homologues (1-19)

R#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
-3	-	-	-	F	-	-	-	-	-	-	-	-	-	-	-	-	Z	-	-
-2	-	-	-	Q	T	-	-	-	-	-	-	Q	-	-	-	H	G	Z	-
-1	-	-	-	T	E	-	-	-	-	-	-	P	-	-	-	D	D	G	-
1	R	R	R	P	R	R	R	R	R	R	R	L	A	R	R	R	K	R	A
2	P	P	P	P	P	P	P	P	P	P	P	R	A	P	P	P	R	P	A
3	D	D	D	D	D	D	D	D	D	D	D	K	K	D	R	T	D	S	K
4	F	F	F	L	F	F	F	F	F	F	F	L	Y	F	F	F	I	F	Y
5	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
6	L	L	L	Q	L	L	L	L	L	L	L	I	K	E	E	N	R	N	K
7	E	E	E	L	E	E	E	E	E	E	E	L	L	L	L	L	L	L	L
8	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	H	**P**	**P**	**P**	**P**	**P**	**P**	**P**
9	P	P	P	Q	P	P	P	P	P	P	P	R	L	A	A	P	P	A	V
10	Y	Y	Y	A	Y	Y	Y	Y	Y	Y	Y	N	R	E	E	E	E	E	R
11	T	T	T	R	T	T	T	T	T	T	T	P	I	T	T	S	Q	T	Y
12	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**
13	P	P	P	P	P	P	P	P	P	P	P	R	P	L	L	R	P	P	P
14	**C**	T	A	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
15	K	K	K	K	K	V	G	A	L	I	K	Y	K	K	K	R	K	K	K
16	A	A	A	A	A	A	A	A	A	A	A	Q	R	A	A	G	G	A	K
17	R	R	R	A	A	R	R	R	R	R	R	K	K	Y	R	H	R	S	K
18	I	I	I	L	M	I	I	I	I	I	I	I	I	I	I	L	L	I	F
19	I	I	I	L	I	I	I	I	I	I	I	P	P	R	R	R	P	R	P
20	R	R	R	R	R	R	R	R	R	R	R	A	S	S	S	R	R	Q	S
21	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	F	F	F	F	I	Y	Y	F
22	F	F	F	F	F	F	F	F	F	F	F	Y	Y	H	H	Y	F	Y	Y
23	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
24	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	K	**N**	**N**	**N**	**N**	**N**	**N**
25	A	A	A	S	A	A	A	A	A	A	A	Q	W	L	R	L	P	S	W
26	K	K	K	T	K	K	K	K	K	K	K	K	K	A	A	E	A	K	K
27	A	A	A	S	A	A	A	A	A	A	A	K	A	A	A	S	S	S	A
28	G	G	G	N	G	G	G	G	G	G	G	K	K	Q	Q	N	R	G	K
29	L	L	L	A	F	L	L	L	L	L	L	Q	Q	Q	Q	K	M	G	Q
30	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**

R#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
31	Q	Q	Q	E	E	Q	Q	Q	Q	Q	Q	E	L	L	L	K	E	Q	L
32	T	T	T	P	T	T	T	T	T	T	T	G	P	Q	E	V	S	Q	P
33	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**
34	V	V	V	T	V	V	V	V	V	V	V	T	D	I	I	F	I	I	N
35	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	W	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
36	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	S	S	**G**	**G**	**G**	**G**	**G**	S
37	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**

38	**C**	T	A	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
39	R	R	R	Q	R	R	R	R	R	R	R	G	G	G	G	G	K	R	G
40	A	A	A	G	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G
41	K	K	K	N	K	K	K	K	K	K	K	N	N	N	N	N	N	N	N
42	R	R	R	N	S	R	R	R	R	R	R	S	A	A	A	A	K	Q	A
43	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
44	N	N	N	N	N	N	N	N	N	N	N	R	R	R	R	N	N	R	R
45	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**
46	K	K	K	E	K	K	K	K	K	K	K	K	K	K	K	E	K	D	K
47	S	S	S	T	S	S	S	S	S	S	S	T	T	T	T	T	T	T	T
48	A	A	A	T	A	A	A	A	A	A	A	I	I	I	I	R	K	T	I
49	E	E	E	E	E	E	E	E	E	E	E	E	E	D	D	D	A	Q	E
50	D	D	D	M	D	D	D	D	D	D	D	E	E	E	E	E	E	Q	E
51	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
52	M	M	M	L	M	M	M	M	M	M	E	R	R	R	H	R	V	Q	R
53	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	E	R	G	R
54	T	T	T	I	T	T	T	T	T	T	T	T	T	T	T	T	A	V	T
55	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
56	G	G	G	E	G	G	G	G	G	G	G	I	V	V	V	G	R	V	V
57	G	G	G	P	G	G	G	G	G	G	G	R	G	G	G	G	P	–	G
58	A	A	A	P	A	A	A	A	A	A	A	K	–	–	–	K	P	–	–
59	–	–	–	Q	–	–	–	–	–	–	–	–	–	–	–	–	E	–	–
60	–	–	–	Q	–	–	–	–	–	–	–	–	–	–	–	–	R	–	–
61	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–	P	–	–
62	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
63	–	–	–	K	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
64	–	–	–	S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

R#	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
-5	-	-	-	-	-	-	-	-	-	-	-	-	-	D	-	-
-4	-	-	-	-	-	-	-	-	-	-	-	-	-	E	-	-
-3	-	-	-	-	-	-	-	-	-	-	-	-	T	P		
-2	Z	-	L	Z	R	K	-	-	-	R	R	-	E	T	-	-
-1	P	-	Q	D	D	N	-	-	-	Q	K	-	R	T	-	-
1	R	R	H	H	R	R	I	K	T	R	R	R	G	D	K	T
2	R	P	R	P	P	P	N	E	V	H	H	P	F	L	A	V
3	K	Y	T	K	K	T	G	D	A	R	P	D	L	P	D	E
4	L	A	F	F	F	F	D	S	A	D	D	F	D	I	S	A
5	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
6	I	E	K	Y	Y	N	E	Q	N	D	D	L	T	E	Q	N
7	L	L	L	L	L	L	L	L	L	K	K	E	S	Q	L	L
8	H	I	**P**	**P**	**P**	L	**P**	G	**P**	**P**	**P**	**P**	**P**	A	D	**P**
9	R	V	A	A	A	P	K	Y	V	P	P	P	P	FG	Y	I
10	N	A	E	D	D	E	V	S	I	D	D	Y	V	D	S	V
11	P	A	P	P	P	T	V	A	R	K	T	T	T	A	Q	Q
12	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	K	**G**	**G**	**G**	**G**	**G**
13	R	P	P	R	R	R	P	P	P	N	I	P	P	L	P	P
14	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
15	Y	M	K	K	L	N	R	M	R	-	-	K	R	F	L	R
16	D	F	A	A	A	A	A	G	A	G	Q	A	A	G	G	A
17	K	F	S	H	Y	L	R	M	F	P	T	K	G	Y	L	F
18	I	I	I	I	M	I	F	T	I	V	V	M	F	M	F	I
19	P	S	P	P	P	P	P	S	Q	R	R	I	K	K	K	Q
20	A	A	A	R	R	A	R	R	L	A	A	R	R	L	R	L
21	F	F	F	F	F	F	Y	Y	W	F	F	Y	Y	Y	Y	W
22	Y	Y	Y	Y	Y	Y	Y	F	A	Y	Y	F	N	S	F	A
23	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	F	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	F
24	**N**	S	**N**	D	**N**	**N**	**N**	**N**	D	D	K	**N**	**N**	**N**	**N**	D
25	Q	K	W	S	P	S	S	G	A	T	P	A	T	Q	G	A
26	K	G	A	A	A	H	S	T	V	R	S	K	R	E	T	V
27	K	A	A	S	S	L	S	S	K	L	A	A	T	T	S	K
28	K	N	K	N	N	H	K	M	G	K	K	G	K	K	M	G

R#	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
29	Q	K	K	K	K	K	R	A	K	T	R	F	Q	N	A	K
30	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
31	E	Y	Q	N	E	Q	E	E	V	K	V	E	E	E	E	V
32	R	P	L	K	K	K	K	T	L	A	Q	T	P	E	T	R
33	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**
34	D	T	H	I	I	N	I	Q	P	Q	R	V	K	I	L	S
35	W	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
36	S	S	**G**	**G**	**G**	**G**	**G**	**G**	**G**	R	**G**	**G**	**G**	**G**	**G**	**G**
37	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**	**G**
38	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
39	G	R	K	P	R	G	G	M	Q	D	D	K	K	Q	M	K
40	G	G	G	G	G	G	G	G	G	G	G	A	G	G	G	G
41	N	N	N	N	N	N	N	N	N	D	D	K	N	N	N	N
42	S	A	A	A	A	A	A	G	G	H	H	S	G	D	L	G
43	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	G	G	**N**	**N**	**N**	**N**	**N**

R#	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
44	R	R	R	N	N	N	N	N	K	N	N	N	R	R	N	K
45	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	Y	**F**	**F**	**F**
46	K	K	S	K	K	K	H	V	Y	K	K	R	K	S	L	Y
47	T	T	T	T	T	T	T	T	S	T	S	S	S	T	S	S
48	I	I	I	W	W	I	L	E	E	E	D	A	E	L	Q	Q
49	E	E	E	D	D	D	E	K	K	T	H	E	Q	A	K	K
50	E	E	K	E	E	E	E	E	E	L	L	D	D	E	E	E
51	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
52	R	R	R	R	R	Q	E	L	R	R	R	M	L	E	L	K
53	R	R	H	Q	H	R	K	Q	E	C	C	R	D	Q	Q	E
54	T	T	A	T	T	T	V	T	Y	E	E	T	A	K	T	Y
55	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**C**
56	I	V	V	G	V	A	G	R	G	L	E	G	S	I	R	G
57	G	V	G	A	A	A	V	-	V	V	L	G	G	N	-	I
58	-	-	-	S	S	K	R	-	P	Y	Y	A	F	-	-	P
59	-	-	-	A	G	Y	S	-	G	P	R	-	-	-	-	G
60	-	-	-	-	I	G	-	-	D	-	-	-	-	-	-	E
61	-	-	-	-	-	-	-	-	E	-	-	-	-	-	-	A

Table ~~13~~ 5, continued (Homologues 36-40)

R#	36	37	38	39	40
-5	-	-	-	-	-
-4	-	-	-	-	-
-3	-	-	-	-	-
-2	-	-	-	-	-
-1	-	Z	-	-	-
1	R	R	R	R	R
2	P	P	P	P	P
3	D	D	D	D	D
4	F	F	F	F	F
5	**C**	**C**	**C**	**C**	**C**
6	L	L	L	L	L
7	E	E	E	E	E
8	**P**	**P**	**P**	**P**	**P**
9	P	P	P	P	P
10	Y	Y	Y	Y	Y
11	T	T	T	T	T
12	**G**	**G**	**G**	**G**	**G**
13	P	P	P	P	P
14	**C**	**C**	**C**	**C**	**C**
15	R	K	K	K	K
16	A	A	A	A	A
17	R	R	R	R	K
18	I	M	I	M	M
19	I	I	I	I	I
20	R	R	R	R	R
21	Y	Y	Y	Y	Y
22	F	F	F	F	F
23	**Y**	**Y**	**Y**	**Y**	**Y**
24	**N**	**N**	**N**	**N**	**N**
25	A	A	A	A	A
26	K	K	K	K	K
27	A	A	A	A	A
28	G	G	G	G	G
29	L	L	L	L	F

30	**C**	**C**	**C**	**C**	**C**
31	Q	Q	Q	Q	E
32	T	P	P	P	T
33	**F**	**F**	**F**	**F**	**F**
34	V	V	V	V	V
35	**Y**	**Y**	**Y**	**Y**	**Y**
36	**G**	**G**	**G**	**G**	**G**
37	**G**	**G**	**G**	**G**	**G**
38	**C**	**C**	**C**	**C**	**C**
39	R	R	R	R	K
40	A	A	A	A	A
41	K	K	K	K	K
42	R	S	R	R	S
43	**N**	**N**	**N**	**N**	**N**

Table ~~13~~ 5, continued

R#	36	37	38	39	40
44	N	N	N	N	N
45	**F**	**F**	**F**	**F**	**F**
46	K	K	K	K	R
47	S	S	S	S	S
48	A	A	S	A	A
49	E	E	E	E	E
50	D	D	D	D	D
51	**C**	**C**	**C**	**C**	**C**
52	E	M	M	M	M
53	R	R	R	R	R
54	T	T	T	T	T
55	**C**	**C**	**C**	**C**	**C**
56	G	G	G	G	G
57	G	G	G	G	G
58	A	A	A	A	A
59	-	-	-	-	-
60	-	-	-	-	-
61	-	-	-	-	-

Legend to Table ~~13~~ 5

1 BPTI SEQ ID NO:87

2 Engineered BPTI From MARK87 SEQ ID NO:88

3 Engineered BPTI From MARK87 SEQ ID NO:89

4 Bovine Colostrum (DUFT85) SEQ ID NO:90

5 Bovine Serum (DUFT85) SEQ ID NO:91

6 Semisynthetic BPTI, TSCH87 SEQ ID NO:92

7 Semisynthetic BPTI, TSCH87 SEQ ID NO:93

8 Semisynthetic BPTI, TSCH87 SEQ ID NO:94

9 Semisynthetic BPTI, TSCH87 SEQ ID NO:95

10 Semisynthetic BPTI, TSCH87 SEQ ID NO:96

11 Engineered BPTI, AUER87 SEQ ID NO:97

12 Dendroaspis polylepis polylepis (Black mamba) venom I(DUFT85) SEQ ID NO:98

13 Dendroaspis polylepis polylepis (Black Mamba) venom K DUFT85) SEQ ID NO:99

14 Hemachatus hemachates (Ringhals Cobra) HHV II (DUFT85) SEQ ID NO:100

15 Naja nivea (Cape cobra) NNV II (DUFT85) SEQ ID NO:101

16 Vipera russelli (Russel's viper) RVV II (TAKA74) SEQ ID NO:102

17 Red sea turtle egg white (DUFT85) SEQ ID NO:103

18 Snail mucus (Helix pomania) (WAGN78) SEQ ID NO:104

19 Dendroaspis angusticeps (Eastern green mamba) C13 S1 C3 toxin (DUFT85) SEQ ID NO:105

20 Dendroaspis angusticeps (Eastern Green Mamba) C13 S2 C3 toxin (DUFT85) SEQ ID NO:106

21 Dendroaspis polylepis polylepes (Black mamba) B toxin (DUFT85) SEQ ID NO:107

22 Dendroaspis polylepis polylepes (Black Mamba) E toxin (DUFT85) SEQ ID NO:108

23 Vipera ammodytes TI toxin (DUFT85) SEQ ID NO:109

24 Vipera ammodytes CTI toxin (DUFT85) SEQ ID NO:110

25 Bungarus fasciatus VIII B toxin (DUFT85) SEQ ID NO:111

26 Anemonia sulcata (sea anemone) 5 II (DUFT85) SEQ ID NO:112

27 Homo sapiens HI-8e "inactive" domain (DUFT85) SEQ ID NO:113

28 Homo sapiens HI-8t "active" domain (DUFT85) SEQ ID NO:114

29 beta bungarotoxin B1 (DUFT85) SEQ ID NO:115

30 beta bungarotoxin B2 (DUFT85) SEQ ID NO:116

31 Bovine spleen TI II (FIOR85) SEQ ID NO:117

32 Tachypleus tridentatus (Horseshoe crab) hemocyte inhibitor (NAKA87) SEQ ID NO:118

33 Bombyx mori (silkworm) SCI-III (SASA84) SEQ ID NO:119

34 Bos taurus (inactive) BI-14 SEQ ID NO:120

35 Bos taurus (active) BI-8 SEQ ID NO:121

36:Engineered BPTI (KR15, ME52) SEQ ID NO:122: Auerswald '88, Biol Chem Hoppe-Seyler, 369 Supplement, pp27-35.

37:Isoaprotinin G-1 SEQ ID NO:123: Siekmann, Wenzel, Schroder, and Tschesche '88, Biol Chem Hoppe-Seyler, 369:157-163.

38:Isoaprotinin 2 SEQ ID NO:124: Siekmann, Wenzel, Schroder, and Tschesche '88, Biol Chem Hoppe-Seyler, 369:157-163.

39:Isoaprotinin G-2 SEQ ID NO:125: Siekmann, Wenzel, Schroder, and Tschesche '88, Biol Chem Hoppe-Seyler, 369:157-163.

40:Isoaprotinin 1 SEQ ID NO:126: Siekmann, Wenzel, Schroder, and Tschesche '88, Biol Chem Hoppe-Seyler, 369:157-163.

Notes :

a) both beta bungarotoxins have residue 15 deleted.

b) B. mori has an extra residue between C5 and C14; we have assigned F and G to residue 9.

c) all natural proteins have C at 5, 14, 30, 38, 50, & 55.

d) all homologues have F33 and G37.

e) extra C's in bungarotoxins form interchain cystine bridges

Please replace Table 30 beginning on page 67 to page 68 with the following amended Table:

Tables

Table ~~30~~ 6: *IIIsp::bpti::~~mautre~~matureIII(initial fragment)* fusion gene.

The DNA sequence has SEQ ID NO. 001; Amino-acid sequence has SEQ ID NO. 002. The DNA is linear and is shown on the lines that do not begin with "!". The DNA encoding mature III is identical to the DNA found in M13mp18. The amino-acid sequence is processed *in vivo* and disulfide bonds form.

```
!  SEQ ID NO. 002      m   k   k   l   l   f   a   I   p   l
!                      1   2   3   4   5   6   7   8   9  10
   SEQ ID NO. 001 5'-gtg aaa aaa tta tta ttc gca att cct tta
!                   |<---- gene III signal peptide --------
!
!                                          ┌ cleavage site
!                                          ↓
!      v   v   p   f   y   s   G   A
!     11  12  13  14  15  16  17  18
      gtt gtt cct ttc tat tct GGc Gcc
!     ------------------------------>|
!
!                  | R | P | D | F | C | L | E |
!                  | 19| 20| 21| 22| 23| 24| 25|
                   |CGT|CCG|GAT|TTC|TGT|CTC|GAG|-
!  M13/BPTI Jnct   ↑  |AccIII|          | XhoI |  (& AvaI)!
!
!  | P | P | Y | T | G | P | C | K | A | R |
!  | 26| 27| 28| 29| 30| 31| 32| 33| 34| 35|
   |CCA|CCA|TAC|ACT|GGG|CCC|TGC|AAA|GCG|CGC|-
!      | PflMI      |      ||       |BssHII |
!                   | ApaI  ||
!                   | DraII  | = PssI
!
!  | I | I | R | Y | F | Y | N | A | K | A |
!  | 36| 37| 38| 39| 40| 41| 42| 43| 44| 45|
   |ATC|ATC|CGC|TAT|TTC|TAC|AAT|GCT|AAA|GC |-
!
```

```
! | G | L | C | Q | T | F | V | Y | G | G |
! | 46| 47| 48| 49| 50| 51| 52| 53| 54| 55|
 A|GGC|CTG|TGC|CAG|ACC|TTT|GTA|TAC|GGT|GGT|-
!| StuI |                  | XcaI  |( & AccI)
!

! | C | R | A | K | R | N | N | F | K |
! | 56| 57| 58| 59| 60| 61| 62| 63| 64|
  |TGC|CGT|GCT|AAG|CGT|AAC|AAC|TTT|AAA|-
!         | EspI   |
!
! | S | A | E | D | C | M | R | T | C | G |
! | 65| 66| 67| 68| 69| 70| 71| 72| 73| 74|
  |TCG|GCC|GAA|GAT|TGC|ATG|CGT|ACC|TGC|GGT|-
!   |XmaIII|        | SphI |
!
!         BPTI/M13 boundary
!         ↓
! | G | A | A   E    (Residue numbers of mature III have had
! | 75| 76|119 120    118 added to the usual residue numbers.)
  |GGC|GCC|gct gaa-
! | NarI  | (& KasI)
!
! 121 122 123 124 125 126 127 128 129 130 131 132 133 134
!  T   V   E   S   C   L   A   K   P   H   T   E   N   S ...
  act gtt gaa agt tgt tta gca aaa ccc cat aca gaa aat tca...
!
```

The remainder of the gene is identical to the corresponding part of *iii* in M13 mp18.

Please replace Table 35 beginning on page 69 to page 70 with the following amended Table:

Table ~~35~~ 7: *IIIsp::itiD1::matureIII* fusion gene.

DNA has SEQ ID NO. 003; amino-acid sequence has SEQ ID NO. 004. The DNA is a linear segment and the amino-acid sequence is a protein that is processed *in vivo* and which contains disulfides.

```
   SEQ ID NO. 004
    m   k   k   l   l   f   a   I   p   l   v   v   p   f   y
   -18 -17 -16 -15 -14 -13 -12 -11 -10  -9  -8  -7  -6  -5  -4
5'-gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
   SEQ ID NO. 003
  |<---- gene III signal peptide ----------------------------

                ┌ cleavage site
    s   G   A   K   E   D   S   C   Q   L   G   Y   S   A   G
   -3  -2  -1   1   2   3   4   5   6   7   8   9   10  11  12
   tct GGc Gcc aaa gaa gaC tcT tGC CAG CTG GGC tac tCG GCC Ggt
    ---------->|                  | BglI       |      | EagI |
       | KasI |

   13  14  15  16  17  18  19  20  21  22  23  24  25  26
    P   C   M   G   M   T   S   R   Y   F   Y   N   G   T
  ccc tgc atg gga atg acc agc agg tat ttc tat aat ggt aca

   27  28  29  30  31  32  33  34  35  36  37  38  39  40  41
    S   M   A   C   E   T   F   Q   Y   G   G   C   M   G   N
  tCC ATG Gcc tgt gag act ttc cag tac ggc ggc tgc atg ggc aac
   | NcoI |
   | StyI |

   42  43  44  45  46  47  48  49  50  51  52  53  54  55  56
    G   N   N   F   V   T   E   K   E   C   L   Q   T   C   R
  ggt aac aac ttc gtc aca gaa aag gag tgt CTG CAG acc tgc cga
                                        | PstI |

   57  58   101 102 119 120
    T   V     g   a   A   E
  act gtg   ggc gcc gct gaa
```

BbeI
NarI
KasI

(Residue numbers of mature III have had 118 added to the usual residue numbers.)

```
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
 T   V   E   S   C   L   A   K   P   H   T   E   N   S   F ..
act gtt gaa agt tgt tta gca aaa ccc cat aca gaa aat tca ttt..
```

The remainder of the gene is identical to the corresponding part of gene *iii* in phage M13mp18.

Please replace Table 55 on page 71 with the following amended Table:

Table ~~55~~ 8: Affinity Classes of ITI-D1-derived hNE inhibitors

Affinity Class	Estimated K_D	Fraction of Input bound	pH Elution Maximum	Protein
WEAK	K_D > 10 nM	<0.005%	> 6.0	ITI-D1
MODERATE	1 to 10 nM	0.01% to 0.03%	5.5 to 5.0	BITI ITI-D1E7
STRONG	10 to 1000 pM	0.03% to 0.06%	5.0 to 4.5	BITI-E7 BITI-E7-1222 AMINO1 AMINO2 MUTP1
VERY STRONG	< 10 pM	> 0.1%	≤ 4.0	BITI-E7-141 MUTT26A MUTQE MUT1619

Please replace Table 65 beginning on page 72 to page 73 with the following amended Table:

Table 65: Definition of Class A, B and C mutations in PCT/US92/01501.

Classes:	A	No major effect expected if molecular charge stays in range -1 to +1.
	B	Major effects not expected, but are more likely than in "A".
	C	Residue in the binding interface; any change must be tested.
	X	No substitution allowed.

Res. Id.	EpiNE1	Substitutions	Class
1	R	any	A
2	P	any	A
3	D	any	A
4	F	Y, W, L	B
5	C	C	X
6	L	non-proline	A
7	E	L, S, T, D, N, K, R	A
8	P	any	A
9	P	any	A
10	Y	non-proline prefr'd	B
11	T	any	C
12	G	must be G	X
13	P	any	C
14	C	C strongly preferred, any non-proline	C
15	I	V, A	C
16	A		C
17	F	L, I, M, Y, W, H, V	C
18	F	Y, W, H	C
19	P	any	C
20	R	non-proline prefr'd	C
21	Y	F & Y most prefr'd; W, I, L prefr'd; M, V allowed	C
22	F	Y & F most prefr'd; non-proline prefr'd	Y, F B
23	Y	Y & F strongly prefr'd	F ,Y B
24	N	non-proline prefr'd	A
25	A	any	A
26	K	any	A

27	A	any	A
28	G	non-proline prefr'd	A
29	L	non-proline prefr'd	A
30	C	must be C	X
31	Q	non-proline prefr'd	B
32	T	non-proline prefr'd	B
33	F	F very strongly prefr'd; Y possible	X
34	V	any	C
35	Y	Y most prefr'd; W prefr'd; F allowed	B

Res. Id.	EpiNE1	Substitutions	Class
36	G	G strongly prefr'd; S, A prefr'd;	C
37	G	must be G so long as 38 is C	X
38	C	C strongly prefr'd	X
39	M	any	C
40	G	A,S,N,D,T,P	C
41	N	K,Q,S,D,R,T,A,E	C
42	G	any	C
43	N	must be N	X
44	N	S,K,R,T,Q,D,E	B
45	F	Y	B
46	K	any non-proline	B
47	ST, N, A, G		B
48	A	any	B
49	E	any	A
50	D	any	A
51	C	must be C	X
52	M	any	A
53	R	any	A
54	T	any	A
55	C	must be C	X
56	G	any	A
57	G	any	A
58	A	any	A

prefr'd stands for preferred.

Please replace Table 100 beginning on page 74 to page 80 with the following amended Table:

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
Consensus Kunitz Domain	RPDFCLLPA-ETGPCRAMIPRFYYNAKSGKCEPFIYGGCGGNA--NNFKTEEECRRTCGGA 1 3 5 7 9 2 4 6 8 10		005
BPTI (Genebank P00974)	RPDFCLEPP-YTGPCKARIIRYFYNAKAGLCQTFVYGGCRAKR-NNFKSAEDCMRTCGGA	BPTI	006
EPI-HNE-1 =EpiNE1	rpdfclepp-ytgpcIaFFPryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	007
EPI-HNE-2	EAEArpdfclepp-ytgpcIaFFPryfynakaglcqtfvyggcMGNG--nnfksaedcmrtcgga	BPTI	008
EpiNE7	rpdfclepp-ytgpcVaMFPryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	009
EpiNE3	rpdfclepp-ytgpcVGFFSryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	010
EpiNE6	rpdfclepp-ytgpcVGFFQryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	011
EpiNE4	rpdfclepp-ytgpcVaIFPryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	012
EpiNE8	rpdfclepp-ytgpcVaFFKrsfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	013
EpiNE5	rpdfclepp-ytgpcIaFFQryfynakaglcqtfvyggcMGNG-nnfksaedcmrtcgga	BPTI	014

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
EpiNE2	rpdfclepp-ytgpcIaLFKryfynakaglcqtfvyggcMGNG—nnfksaedcmrtcgga	BPTI	015
ITI-D1 (Genebank P02760)	KEDSCQLGY-SAGPCMGMTSRYFYNGTSMACETFQYGGCMGNG—NNFVTEKDCLQTCRTV	ITI-D1	016
BITI-E7-141	RPdFcqlgy-sagpcVAmFPryfyngtsmacQtfVyggcmgng—nnfvtekdclqtcrga	ITI-D1	017
MUTT26A	RPdFcqlgy-sagpcVAmFPryfyngAsmacQtfVyggcmgng—nnfvtekdclqtcrga	ITI-D1	018
MUTQE	RPdFcqlgy-sagpcVAmFPryfyngtsmacetfVyggcmgng—nnfvtekdclqtcrga	ITI-D1	019
MUT1619	RPdFcqlgy-sagpcVgmFsryfyngtsmacQtfVyggcmgng—nnfvtekdclqtcrga	IDI-D1	020
ITI-D1E7	kedscqlgy-sagpcVAmFPryfyngtsmacetfqyggcmgng—nnfvtekdclqtcrga	ITI-D1	021
AMINO1	kedFcqlgy-sagpcVAmFPryfyngtsmacetfqyggcmgng—nnfvtekdclqtcrga	ITI-D1	022
AMINO2	kPdscqlgy-sagpcVAmFPryfyngtsmacetfqyggcmgng—nnfvtekdclqtcrga	ITI-D1	023
MUTP1	RPdFcqlgy-sagpcIgmFsryfyngtsmacetfqyggcmgng—nnfvtekdclqtcrga	ITI-D1	024
ITI-D2 (Genebank P02760)	TVAACNLPI-VRGPCRAFIQLWAFDAVKGKCVLFPYGGCQGNG—NKFYSEKECREYCGVP	ITI-D2	025
EPI-HNE-3	aacnlpi-vrgpcIafFPRwafdavkgkcvlfpyggcqgng—nkfysekecreycgvp	ITI-D2	026

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
EPI-HNE-4	Eacnlpi-vrgpcIafFPRwafdavkgkcvlfpyggcqgng-nkfysekecreycgvp	ITI-D2	027
App-I (NCBI 105306)	VREVCSEQA-ETGPCRAMISRWYFDVTEGKCAPFFYGGCGGNR-NNFDTEEYCMAVCGSA		028
DPI.1.1	vrevcseqa-YtgpcIaFFPrYyfdvtegkcQTfVyggcMgnG-nnfdteeycmavcgsa	APP-I	029
DPI.1.2	vrevcseqa-etgpcIamFsrwyfdvtegkcapfVyggcggnr-nnfdteeycmavcgsa	App-I	030
DPI.1.3	vrevcseqa-etgpcIaFFsrwyfdvtegkcaTfVyggcMgnr-nnfdteeycmavcgsa	App-I	031
TFPI2-D1 (SPRE94)	NAEICLLPL-DYGPCRALLLRYYYDRYTQSCRQFLYGGCEGNA-NNFYTWEACDDACWRI		032
DPI.2.1	naeicllpl-YTgpcIaFFPryyydrytqscQTfVyggcMgna-nnfytweacddacwri	TFPI2-D1	033
DPI.2.2	naeicllpl-dygpcIalFlryyydrytqscrqfVyggcegna-nnfytweacddacwri	TFPI2-D1	034
DPI.2.3	naeicllpl-dTgpcIaFFlryyydrytqscQTfVyggcMgna-nnfytweacddacwri	TFPI2-D1	035
TFPI2-D2 (SPRE94)	VPKVCRLQVSVDDQCEGSTEKYFFNLSSMTCEKFFSGGCHRNRIENRFPDEATCMGFCAPK		036
DPI.3.1	vpkvcrlqv-vRGPcIAFFPRWffnlssmtcVLfPYggcQGnG-nrfpdeatcmgfcapk		037
DPI.3.2	vpkvcrlqvsvddqcIgsFekyffnlAsmtceTfVsggchrnrienrfpdeatcmgfcapk	TFPI2-D2	038
DPI.3.3	vpkvcrlqv-vAGPcIgFFKRyffAlssmtceTfVsggchrnr-nrfpdeatcmgfcapk	TFPI2-D2	039
TFPI2-D3	ipsfcyspk-deglcsanvtryyfnpryrtcdaftytgcggnd-nnfvsredckracaka		040

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
(SPRE94)			
DPI.4.1	ipsfcyspk-SAgPcVaMFPryyfnpryrtcETfVyGgcMgnG—nnfvsredckracaka	TFPI2-D3	041
DPI.4.2	ipsfcyspk-deglcIaFFtryyfnpryrtcdaftytgcggnd—nnfvsredckracaka	TFPI2-D3	042
DPI.4.3	ipsfcyspk-dTgPcIaFFtryyfnpryrtcdTfVyGgcggnd—nnfvsredckracaka	TFPI2-D3	043
LACI-D1 (Genebank P10646)	mhsfcafka-ddgpckaimkrfffniftrqceefiyggcegnq--nrfesleeckkmctrd		044
DPI.5.1	mhsfcafka-SAgpcVaMFPrYffniftrqceTfVyggcMgnG—nrfesleeckkmctrd	LACI-D1	045
DPI.5.2	mhsfcafka-ddgpcIaiFkrfffniftrqceefiyggcegnq—nrfesleeckkmctrd	LACI-D1	046
DPI.5.3	mhsfcafka-YTgpcIaFFkrfffniftrqceTfiyggcegnq—nrfesleeckkmctrd	LACI-D1	047
LACI-D2 (Genebank P10646)	KPDFCFLEE-DPGICRGYITRYFYNNQTKQCERFKYGGCLGNM—NNFETLEECKNICEDG		048
DPI.6.1	kpdfcflee-SAgPcVAMFPryfynnqtkqceTfVyggcMgnG—nnfetleecknicedg	LACI-D2	049
DPI.6.2	kpdfcflee-dpgicVgyFtryfynnqtkqcerfkyggclgnm—nnfetleecknicedg	LACI-D2	050
DPI.6.3	kpdfcflee-dpgicVgFFtryfynnqtkqcerfVyggclgnm—nnfetleecknicedg	LACI-D2	051
DPI.6.4	kpdfcflee-dpgicVgFFtryfynAqtkqcerfVyggclgnm—nnfetleecknicedg	LACI-D2	052
DPI.6.5	kpdfcflee-dpgPcVgFFQryfynAqtkqcerfVyggcQgnm—nnfetleecknicedg	LACI-D2	053

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
DPI.6.6	kpdfcflee-dpgPcVgFFtryfynnqtkqcerfVyggcQgnm-nnfetleecknicedg	LACI-D2	054
DPI.6.7	kpdfcflee-dpgPcIgFFPryfynnqtkqcerfVyggcQgnm-nnfetleecknicedg	LACI-D2	055
LACI-D3 (Genebank P10646)	GPSWCLTPA-DRGLCRANENRFYYNSVIGKCRPFKYSGCGGNE-NNFTSKQECLRACKKG		056
DPI.7.1	gpswcltpa-VrgPcIaFFPrWyynsvigkcVLfPyGgcQgnG-nnftskqeclrackkg	LACI-D3	057
DPI.7.2	gpswcltpa-drglcVanFnrfyynsvigkcrpfkysgcggne-nnftskqeclrackkg	LACI-D3	058
DPI.7.3	gpswcltpa-drglcVaFFnrfyynsvigkcrpfkysgcggne-nnfKskqeclrackkg	LACI-D3	059
DPI.7.4	gpswcltpa-VrgPcVaFFnrfyynsvigkcrpfkyGgcggne-nnfKskqeclrackkg	LACI-D3	060
DPI.7.5	gpswcltpa-drgPcIaFFPrWyynsvigkcQTfVyGgcggne-nnfAskqeclrackkg	LACI-D3	061
A3 collagen (WO93/ 14119)	ETDICKLPK-DEGTCRDFILKWYYDPNTKSCARFWYGGCGGNE-NKFGSQKECEKVCAPV		062
DPI.8.1	etdicklpk-VRgPcIAfFPRwyydpntkscVLfPyggcQgnG-nkfgsqkecekvcapv	A3	063
DPI.8.2	etdicklpk-degtcIAfFlkwyydpntkscarfVyggcggne-nkfgsqkecekvcapv	A3 collagen	064
DPI.8.3	etdicklpk-degPcIAfFlRwyydpntkscarfVyggcggne-nkfgsqkecekvcapv	A3	065
HKI B9	LPNVCAFPM-EKGPCQTYMTRWFFNFETGECELFAYGGCGGNS-NNFLRKEKCEKFCKFT		066

Table ~~100~~ 10: Sequences of Kunitz domains

Name	Sequence 1111111111222222222233333333333444 4444444555555555 123456789a012345678901234567890123456789012ab3456789012345678	Parental domain	Seq Id No.
Domain (NORR93)			
DPI.9.1	lpnvcafpm-VRgpcIAFFPrwffnfetgecVlfVyggcQgnG-nnflrkekcekfckft	HKI B9	067
DPI.9.2	lpnvcafpm-ekgpcIAyFtrwffnfetgecelfayggcggns-nnflrkekcekfckft	HKI B9	068
DPI.9.3	lpnvcafpm-ekgpcIAyFPrwffnfetgecVlfVyggcggns-nnflrkekcekfckft	HKI B9	069

Sequences listed in Table ~~100~~ 10 that strongly inhibit hNE are EPI-HNE-1(=EpiNE1), EPI-HNE-2, EpiNE7, EpiNE3, EpiNE6, EpiNE4, EpiNE8, EpiNE5, EpiNE2, BITI-E7-141, MUTT26A, MUTQE, MUT1619, ITI-D1E7, AMINO1, AMINO2, MUTP1, and EPI-HNE-3, and EPI-HNE-4. Sequences listed in Table 100 that are highly likely to strongly inhibit hNE are DPI.1.1, DPI.1.2, DPI.1.3, DPI.2.1, DPI.2.2, DPI.2.3, DPI.3.1, DPI.3.2, DPI.3.3, DPI.4.1, DPI.4.2, DPI.4.3, DPI.5.1, DPI.5.2, DPI.5.3, DPI.6.1, DPI.6.2, DPI.6.3, DPI.6.4, DPI.6.5, DPI.6.6, DPI.6.7, DPI.7.1, DPI.7.2, DPI.7.3, DPI.7.4, DPI.7.5, DPI.8.1, DPI.8.2, DPI.8.3, DPI.9.1, DPI.9.2, and DPI.9.3. Human Kunitz domains listed in Table 100: ITI-D1, ITI-D2, App-I, TFPI2-D1, TFPI2-D2, TFPI2-D3, LACI-D1, LACI-D2, LACI-D3, A3 collagen Kunitz domain, and HKI B9 Domain.

Please replace Table 111 beginning on page 80 to page 81 with the following amended Table:

Table ~~111~~ 11: Restriction sites in plasmid pHIL-D2

pHIL-D2, 93-01-02 Ngene = 8157

Non-cutters

AflII	ApaI	AscI	AvaI	AvrII	BamHI	BglII
Bsp120I	BsrGI	BssHII	BstEII	FseI	MluI	NruI
PacI	PmlI	RsrII	SacII	SexAI	SfiI	SgfI
SnaBI	SpeI	Sse8387I	XhoI(PaeR7I)		XmaI(SmaI)	

Cutters

AatII GACGTc	1	5498	
AflIII Acrygt	1	7746	
AgeI Accggt	1	1009	
BlpI GCtnagc	1	597	
BspEI(BspMII,AccIII) Tccgga	1	3551	
BspMI gcaggt	1	4140	
Bst1107I GTAtac	1	7975	
BstBI(AsuII) TTcgaa	2	945	4780
Bsu36I CCtnagg	1	1796	
Ecl136I GAGctc	1	216	
EcoRI Gaattc	1	956	
EspI(Bpu1102I) GCtnagc	1	597	
HpaI GTTaac	1	1845	
NcoI Ccatgg	1	3339	
NdeI CAtatg	1	7924	
NsiI(Ppu10I) ATGCAt	1	684	
PflMI CCANNNNntgg	1	196	

PmeI GTTTaaac	1	420
PstI CTGCAg	1	6175
PvuI CGATcg	1	6049
SapI gaagagc	1	7863
SacI GAGCTc	1	216
SalI Gtcgac	1	2885
ScaI AGTact	1	5938
SphI GCATGc	1	4436
StuI AGGcct	1	2968
SwaI ATTTaaat	1	6532
Tth111I GACNnngtc	1	7999
XbaI Tctaga	1	1741
XcmI CCANNNNNnnnntgg	1	711

Aox1 5'	1 to about 950
Aox1 3'	950 to about 1250
His4	1700 to about 4200
Aox1 3'	4500 to 5400
bla	5600 to 6400
f1 ori	6500 to 6900

Please replace Tables 207 and 208 on page 82 with the following amended Table:

TABLES ~~207-208~~ 12-13 (merged)

SEQUENCES OF THE EpiNE CLONES IN THE P1 REGION

CLONE IDENTIFIERS	SEQUENCE
	1 1 1 1 1 1 1 2 2 3 4 5 6 7 8 9 0 1
BPTI (comp. only)	P C K A R I I R Y (BPTI) (SEQ ID NO:~~6~~132) P C V A M F Q R Y EpiNEα (SEQ ID NO:129)
3, 9, 16, 17, 18, 19	P C V G F F S R Y EpiNE3 (SEQ ID NO:~~10~~133)
6	P C V G F F Q R Y EpiNE6 (SEQ ID NO:~~11~~134)
7, 13, 14, 15, 20	P C V A M F P R Y EpiNE7 (SEQ ID NO:~~9~~135)
4	P C V A I F P R Y EpiNE4 (SEQ ID NO:~~12~~136)
8	P C V A I F K R S EpiNE8 (SEQ ID NO:~~13~~137)
1, 10, 11, 12	P C I A F F P R Y EpiNE1 (SEQ ID NO:~~7~~138)
5	P C I A F F Q R Y EpiNE5 (SEQ ID NO:~~14~~139)
2	P C I A L F K R Y EpiNE2 (SEQ ID NO:~~15~~140)

Note: The DNA sequences encoding these amino acid sequences are set forth in 08/133,031, previously incorporated by reference.

Please replace Table 212 on page 83 with the following amended Table:

TABLE ~~212~~ 14: Fractionation of EpiNE-7 and MA-ITI-D1 phage on hNE beads

	EpiNE-7		MA-ITI-D1	
	pfu	pfu/INPUT	pfu	pfu/INPUT
INPUT	$3.3 \cdot 10^{9}$	1.00	$3.4 \cdot 10^{11}$	1.00
Final TBS-TWEEN Wash	$3.8 \cdot 10^{5}$	$1.2 \cdot 10^{-4}$	$1.8 \cdot 10^{6}$	$5.3 \cdot 10^{-6}$
pH 7.0	$6.2 \cdot 10^{5}$	$1.8 \cdot 10^{-4}$	$1.6 \cdot 10^{6}$	$4.7 \cdot 10^{-6}$
6.0	$1.4 \cdot 10^{6}$	$4.1 \cdot 10^{-4}$	$1.0 \cdot 10^{6}$	$2.9 \cdot 10^{-6}$
5.5	$9.4 \cdot 10^{5}$	$2.8 \cdot 10^{-4}$	$1.6 \cdot 10^{6}$	$4.7 \cdot 10^{-6}$
5.0	$9.5 \cdot 10^{5}$	$2.9 \cdot 10^{-4}$	$3.1 \cdot 10^{5}$	$9.1 \cdot 10^{-7}$
4.5	$1.2 \cdot 10^{6}$	$3.5 \cdot 10^{-4}$	$1.2 \cdot 10^{5}$	$3.5 \cdot 10^{-7}$
4.0	$1.6 \cdot 10^{6}$	$4.8 \cdot 10^{-4}$	$7.2 \cdot 10^{4}$	$2.1 \cdot 10^{-7}$
3.5	$9.5 \cdot 10^{5}$	$2.9 \cdot 10^{-4}$	$4.9 \cdot 10^{4}$	$1.4 \cdot 10^{-7}$
3.0	$6.6 \cdot 10^{5}$	$2.0 \cdot 10^{-4}$	$2.9 \cdot 10^{4}$	$8.5 \cdot 10^{-8}$
2.5	$1.6 \cdot 10^{5}$	$4.8 \cdot 10^{-5}$	$1.4 \cdot 10^{4}$	$4.1 \cdot 10^{-8}$
2.0	$3.0 \cdot 10^{5}$	$9.1 \cdot 10^{-5}$	$1.7 \cdot 10^{4}$	$5.0 \cdot 10^{-8}$
SUM	$6.4 \cdot 10^{6}$	$3 \cdot 10^{-3}$	$5.7 \cdot 10^{6}$	$2 \cdot 10^{-5}$

* SUM is the total pfu (or fraction of input) obtained from all pH elution fractions

Please replace Table 214 on page 84 with the following amended Table:

TABLE ~~214~~ 15: Abbreviated fractionation of display phage on hNE beads

	Display phage			
	EpiNE-7	MA-ITI-D1 2	MA-ITI-D1E7 1	MA-ITI-D1E7 2
INPUT (pfu)	1.00 (1.8×10^9)	1.00 (1.2×10^{10}	1.00 (3.3×10^9)	1.00 (1.1×10^9)
Wash	$6 \cdot 10^{-5}$	$1 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-5}$
pH 7.0	$3 \cdot 10^{-4}$	$1 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$4 \cdot 10^{-5}$
pH 3.5	$3 \cdot 10^{-3}$	$3 \cdot 10^{-6}$	$8 \cdot 10^{-5}$	$8 \cdot 10^{-5}$
pH 2.0	$1 \cdot 10^{-3}$	$1 \cdot 10^{-6}$	$6 \cdot 10^{-6}$	$2 \cdot 10^{-5}$
SUM	$4.3 \cdot 10^{-3}$	$1.4 \cdot 10^{-5}$	$1.1 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$

Each entry is the fraction of input obtained in that component.

SUM is the total fraction of input pfu obtained from all pH elution fractions

Please replace Table 215 on page 85 with the following amended Table:

TABLE ~~215~~ 16: Fractionation of EpiNE-7 and MA-ITI-D1E7 phage on hNE beads

	EpiNE-7		MA-ITI-D1E7	
	Total pfu	Fraction of Input	Total pfu	Fraction of Input
INPUT	$1.8 \cdot 10^9$	1.00	$3.0 \cdot 10^9$	1.00
pH 7.0	$5.2 \cdot 10^5$	$2.9 \cdot 10^{-4}$	$6.4 \cdot 10^4$	$2.1 \cdot 10^{-5}$
pH 6.0	$6.4 \cdot 10^5$	$3.6 \cdot 10^{-4}$	$4.5 \cdot 10^4$	$1.5 \cdot 10^{-5}$
pH 5.5	$7.8 \cdot 10^5$	$4.3 \cdot 10^{-4}$	$5.0 \cdot 10^4$	$1.7 \cdot 10^{-5}$
pH 5.0	$8.4 \cdot 10^5$	$4.7 \cdot 10^{-4}$	$5.2 \cdot 10^4$	$1.7 \cdot 10^{-5}$
pH 4.5	$1.1 \cdot 10^6$	$6.1 \cdot 10^{-4}$	$4.4 \cdot 10^4$	$1.5 \cdot 10^{-5}$
pH 4.0	$1.7 \cdot 10^6$	$9.4 \cdot 10^{-4}$	$2.6 \cdot 10^4$	$8.7 \cdot 10^{-6}$
pH 3.5	$1.1 \cdot 10^6$	$6.1 \cdot 10^{-4}$	$1.3 \cdot 10^4$	$4.3 \cdot 10^{-6}$
pH 3.0	$3.8 \cdot 10^5$	$2.1 \cdot 10^{-4}$	$5.6 \cdot 10^3$	$1.9 \cdot 10^{-6}$
pH 2.5	$2.8 \cdot 10^5$	$1.6 \cdot 10^{-4}$	$4.9 \cdot 10^3$	$1.6 \cdot 10^{-6}$
pH 2.0	$2.9 \cdot 10^5$	$1.6 \cdot 10^{-4}$	$2.2 \cdot 10^3$	$7.3 \cdot 10^{-7}$
SUM	$7.6 \cdot 10^6$	$4.1 \cdot 10^{-3}$	$3.1 \cdot 10^5$	$1.1 \cdot 10^{-4}$

* SUM is the total pfu (or fraction of input) obtained from all pH elution fractions.

Please replace Table 216 on page 86 with the following amended Table:

TABLE ~~216~~ 17: Fractionation of MA-EpiNE-7, MA-BITI and MA-BITI-E7 on hNE beads

	MA-BITI		MA-BITI-E7		MA-EpiNE7	
	pfu	pfu/Input	pfu	pfu/Input	pfu	pfu/Input
INPUT	$2.0 \cdot 10^{10}$	1.00	$6.0 \cdot 10^{9}$	1.00	$1.5 \cdot 10^{9}$	1.00
pH 7.0	$2.4 \cdot 10^{5}$	$1.2 \cdot 10^{-5}$	$2.8 \cdot 10^{5}$	$4.7 \cdot 10^{-5}$	$2.9 \cdot 10^{5}$	$1.9 \cdot 10^{-4}$
6.0	$2.5 \cdot 10^{5}$	$1.2 \cdot 10^{-5}$	$2.8 \cdot 10^{5}$	$4.7 \cdot 10^{-5}$	$3.7 \cdot 10^{5}$	$2.5 \cdot 10^{-4}$
5.0	$9.6 \cdot 10^{4}$	$4.8 \cdot 10^{-6}$	$3.7 \cdot 10^{5}$	$6.2 \cdot 10^{-5}$	$4.9 \cdot 10^{5}$	$3.3 \cdot 10^{-4}$
4.5	$4.4 \cdot 10^{4}$	$2.2 \cdot 10^{-6}$	$3.8 \cdot 10^{5}$	$6.3 \cdot 10^{-5}$	$6.0 \cdot 10^{5}$	$4.0 \cdot 10^{-4}$
4.0	$3.1 \cdot 10^{4}$	$1.6 \cdot 10^{-6}$	$2.4 \cdot 10^{5}$	$4.0 \cdot 10^{-5}$	$6.4 \cdot 10^{5}$	$4.3 \cdot 10^{-4}$
3.5	$8.6 \cdot 10^{4}$	$4.3 \cdot 10^{-6}$	$9.0 \cdot 10^{4}$	$1.5 \cdot 10^{-5}$	$5.0 \cdot 10^{5}$	$3.3 \cdot 10^{-4}$
3.0	$2.2 \cdot 10^{4}$	$1.1 \cdot 10^{-6}$	$8.9 \cdot 10^{4}$	$1.5 \cdot 10^{-5}$	$1.9 \cdot 10^{5}$	$1.3 \cdot 10^{-4}$
2.5	$2.2 \cdot 10^{4}$	$1.1 \cdot 10^{-6}$	$2.3 \cdot 10^{4}$	$3.8 \cdot 10^{-6}$	$7.7 \cdot 10^{4}$	$5.1 \cdot 10^{-5}$
2.0	$7.7 \cdot 10^{3}$	$3.8 \cdot 10^{-7}$	$8.7 \cdot 10^{3}$	$1.4 \cdot 10^{-6}$	$9.7 \cdot 10^{4}$	$6.5 \cdot 10^{-5}$
SUM	$8.0 \cdot 10^{5}$	$3.9 \cdot 10^{-5}$	$1.8 \cdot 10^{6}$	$2.9 \cdot 10^{-4}$	$3.3 \cdot 10^{6}$	$2.2 \cdot 10^{-3}$

* SUM is the total pfu (or fraction of input) obtained from all pH elution fractions

Please replace Table 217 on page 87 with the following amended Table:

TABLE ~~217~~ 18: Fractionation of MA-BITI-E7 and MA-BITI-E7-1222 on hNE beads

	MA-BITI-E7		MA-BITI-E7-1222	
	pfu	pfu/INPUT	pfu	pfu/INPUT
INPUT	$1.3 \cdot 10^{9}$	1.00	$1.2 \cdot 10^{9}$	1.00
pH 7.0	$4.7 \cdot 10^{4}$	$3.6 \cdot 10^{-5}$	$4.0 \cdot 10^{4}$	$3.3 \cdot 10^{-5}$
6.0	$5.3 \cdot 10^{4}$	$4.1 \cdot 10^{-5}$	$5.5 \cdot 10^{4}$	$4.6 \cdot 10^{-5}$
5.5	$7.1 \cdot 10^{4}$	$5.5 \cdot 10^{-5}$	$5.4 \cdot 10^{4}$	$4.5 \cdot 10^{-5}$
5.0	$9.0 \cdot 10^{4}$	$6.9 \cdot 10^{-5}$	$6.7 \cdot 10^{4}$	$5.6 \cdot 10^{-5}$
4.5	$6.2 \cdot 10^{4}$	$4.8 \cdot 10^{-5}$	$6.7 \cdot 10^{4}$	$5.6 \cdot 10^{-5}$
4.0	$3.4 \cdot 10^{4}$	$2.6 \cdot 10^{-5}$	$2.7 \cdot 10^{4}$	$2.2 \cdot 10^{-5}$
3.5	$1.8 \cdot 10^{4}$	$1.4 \cdot 10^{-5}$	$2.3 \cdot 10^{4}$	$1.9 \cdot 10^{-5}$
3.0	$2.5 \cdot 10^{3}$	$1.9 \cdot 10^{-6}$	$6.3 \cdot 10^{3}$	$5.2 \cdot 10^{-6}$
2.5	$<1.3 \cdot 10^{3}$	$<1.0 \cdot 10^{-6}$	$<1.3 \cdot 10^{3}$	$<1.0 \cdot 10^{-6}$
2.0	$1.3 \cdot 10^{3}$	$1.0 \cdot 10^{-6}$	$1.3 \cdot 10^{3}$	$1.0 \cdot 10^{-6}$
SUM	$3.8 \cdot 10^{5}$	$2.9 \cdot 10^{-4}$	$3.4 \cdot 10^{5}$	$2.8 \cdot 10^{-4}$

SUM is the total pfu (or fraction of input) obtained from all pH elution fractions

Please replace Table 218 on page 88 with the following amended Table:

TABLE ~~218~~ 19: Fractionation of MA-EpiNE7 and MA-BITI-E7-141 on hNE beads

	MA-EpiNE7		MA-BITI-E7-141	
	pfu	pfu/INPUT	pfu	pfu/INPUT
INPUT	$6.1 \cdot 10^{8}$	1.00	$2.0 \cdot 10^{9}$	1.00
pH 7.0	$5.3 \cdot 10^{4}$	$8.7 \cdot 10^{-5}$	$4.5 \cdot 10^{5}$	$2.2 \cdot 10^{-4}$
6.0	$9.7 \cdot 10^{4}$	$1.6 \cdot 10^{-4}$	$4.4 \cdot 10^{5}$	$2.2 \cdot 10^{-4}$
5.5	$1.1 \cdot 10^{5}$	$1.8 \cdot 10^{-4}$	$4.4 \cdot 10^{5}$	$2.2 \cdot 10^{-4}$
5.0	$1.4 \cdot 10^{5}$	$2.3 \cdot 10^{-4}$	$7.2 \cdot 10^{5}$	$3.6 \cdot 10^{-4}$
4.5	$1.0 \cdot 10^{5}$	$1.6 \cdot 10^{-4}$	$1.3 \cdot 10^{6}$	$6.5 \cdot 10^{-4}$
4.0	$2.0 \cdot 10^{5}$	$3.3 \cdot 10^{-4}$	$1.1 \cdot 10^{6}$	$5.5 \cdot 10^{-4}$
3.5	$9.7 \cdot 10^{4}$	$1.6 \cdot 10^{-4}$	$5.9 \cdot 10^{5}$	$3.0 \cdot 10^{-4}$
3.0	$3.8 \cdot 10^{4}$	$6.2 \cdot 10^{-5}$	$2.3 \cdot 10^{5}$	$1.2 \cdot 10^{-4}$
2.5	$1.3 \cdot 10^{4}$	$2.1 \cdot 10^{-5}$	$1.2 \cdot 10^{5}$	$6.0 \cdot 10^{-5}$
2.0	$1.6 \cdot 10^{4}$	$2.6 \cdot 10^{-5}$	$1.0 \cdot 10^{5}$	$5.0 \cdot 10^{-5}$
SUM	$8.6 \cdot 10^{5}$	$1.4 \cdot 10^{-3}$	$5.5 \cdot 10^{6}$	$2.8 \cdot 10^{-3}$

SUM is the total pfu (or fraction of input) obtained from all pH elution fractions.

Please replace Table 218 on page 89 with the following amended Table:

TABLE ~~219~~ 20: pH Elution Analysis of hNE Binding by BITI-E7-141 Varient Display Phage

Displayed protein	Input	Fraction of Input recovered at pH			Recovery	
	PFU ($x10^9$)	pH7.0	pH3.5 $x10^{-4}$	pH2.0 $x10^{-4}$	Total $x10^{-4}$	Relative
AMINO1 (EE)	0.96	0.24	2.3	0.35	2.9	0.11
AMINO2 (AE)	6.1	0.57	2.1	0.45	3.1	0.12
BITI-E7-1222 (EE)	1.2	0.72	4.0	0.64	5.4	0.21
EpiNE7 (EE)	0.72	0.44	6.4	2.2	9.0	0.35
MUTP1 (AE)	3.9	1.8	9.2	1.2	12.0	0.46
MUT1619 (EE)	0.78	0.82	9.9	0.84	12.0	0.46
MUTQE (AE)	4.7	1.2	16.	5.3	22.0	0.85
MUTT26A (EE)	0.51	2.5	19.0	3.3	25.0	0.96
BITI-E7-141 (AE)	1.7	2.2	18.0	5.4	26.0	1.00
BITI-E7-141 (EE)	0.75	2.1	21.	3.2	26.0	1.00

Notes:

EE Extended pH elution protocol

AE Abbreviated pH elution protocol

Total Total fraction of input = Sum of fractions collected at pH 7.0, pH 3.5, and pH 2.0.

Relative Total fraction of input recovered divided by total fraction of input recovered for BITI-E7-141

Please replace Table 250 beginning on page 90 to page 94 with the following amended Table:

Table ~~250~~ 23: Plasmid pHIL-D2 SEQ ID NO. 070

8157 base pairs. Only one strand is shown, but the DNA exists as double-stranded circular DNA *in vivo*.

```
                1          2          3          4          5
      1234567890 1234567890 1234567890 1234567890 1234567890
    1 AgATCgCggC CgCgATCTAA CATCCAAAgA CgAAAggTTg AATgAAACCT
   51 TTTTgCCATC CgACATCCAC AggTCCATTC TCACACATAA gTgCCAAACg
  101 CAACAggAgg ggATACACTA gCAgCAgACC gTTgCAAACg CAggACCTCC
  151 ACTCCTCTTC TCCTCAACAC CCACTTTTgC CATCgAAAAA CCAgCCCAgT
  201 TATTgggCTT gATTggAgCT CgCTCATTCC AATTCCTTCT ATTAggCTAC
  251 TAACACCATg ACTTTATTAg CCTgTCTATC CTggCCCCCC TggCgAggTC
  301 ATgTTTgTTT ATTTCCgAAT gCAACAAgCT CCgCATTACA CCCgAACATC
  351 ACTCCAgATg AgggCTTTCT gAgTgTgggg TCAAATAgTT TCATgTTCCC
  401 AAATggCCCA AAACTgACAg TTTAAACgCT gTCTTggAAC CTAATATgAC
  451 AAAAgCgTgA TCTCATCCAA gATgAACTAA gTTTggTTCg TTgAAATgCT
  501 AACggCCAgT TggTCAAAAA gAAACTTCCA AAAgTCgCCA TACCgTTTgT
  551 CTTgTTTggT ATTgATTgAC gAATgCTCAA AAATAATCTC ATTAATgCTT
  601 AgCgCAgTCT CTCTATCgCT TCTgAACCCg gTggCACCTg TgCCgAAACg
  651 CAAATggggA AACAACCCgC TTTTTggATg ATTATgCATT gTCCTCCACA
  701 TTgTATgCTT CCAAgATTCT ggTgggAATA CTgCTgATAg CCTAACgTTC
  751 ATgATCAAAA TTTAACTgTT CTAACCCCTA CTTgACAggC AATATATAAA
  801 CAgAAggAAg CTgCCCTgTC TTAAACCTTT TTTTTTATCA TCATTATTAg
  851 CTTACTTTCA TAATTgCgAC TggTTCCAAT TgACAAgCTT TTgATTTTAA
  901 CgACTTTTAA CgACAACTTg AgAAgATCAA AAAACAACTA ATTATTCgAA
                                                    BstBI
  951 ACgAggAATT CgCCTTAgAC ATgACTgTTC CTCAgTTCAA gTTgggCATT
          EcoRI
 1001 ACgAgAAgAC CggTCTTgCT AgATTCTAAT CAAgAggATg TCAgAATgCC
 1051 ATTTgCCTgA gAgATgCAgg CTTCATTTTT gATACTTTTT TATTTgTAAC
 1101 CTATATAgTA TAggATTTTT TTTgTCATTT TgTTTCTTCT CgTACgAgCT
 1151 TgCTCCTgAT CAgCCTATCT CgCAgCTgAT gAATATCTTg TggTAggggT
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1201 TTgggAAAAT CATTCgAgTT TgATgTTTTT CTTggTATTT CCCACTCCTC
1251 TTCAgAgTAC AgAAgATTAA gTgAgAAgTT CgTTTgTgCA AgCTTATCgA
1301 TAAgCTTTAA TgCggTAgTT TATCACAgTT AAATTgCTAA CgCAgTCAgg
1351 CACCgTgTAT gAAATCTAAC AATgCgCTCA TCgTCATCCT CggCACCgTC
1401 ACCCTggATg CTgTAggCAT AggCTTggTT ATgCCggTAC TgCCgggCCT
1451 CTTgCgggAT ATCgTCCATT CCgACAgCAT CgCCAgTCAC TATggCgTgC
1501 TgCTAgCgCT ATATgCgTTg ATgCAATTTC TATgCgCACC CgTTCTCggA

Table ~~250~~ 23, continued

1551 gCACTgTCCg ACCgCTTTgg CCgCCgCCCA gTCCTgCTCg CTTCgCTACT
1601 TggAgCCACT ATCgACTACg CgATCATggC gACCACACCC gTCCTgTggA
1651 TCTATCgAAT CTAAATgTAA gTTAAAATCT CTAAATAATT AAATAAgTCC
1701 CAgTTTCTCC ATACgAACCT TAACAgCATT gCggTgAgCA TCTAgACCTT
1751 CAACAgCAgC CAgATCCATC ACTgCTTggC CAATATgTTT CAgTCCCTCA
1801 ggAgTTACgT CTTgTgAAgT gATgAACTTC TggAAggTTg CAgTgTTAAC
1851 TCCgCTgTAT TgACgggCAT ATCCgTACgT TggCAAAgTg TggTTggTAC
1901 CggAggAgTA ATCTCCACAA CTCTCTggAg AgTAggCACC AACAAACACA
1951 gATCCAgCgT gTTgTACTTg ATCAACATAA gAAgAAgCAT TCTCgATTTg
2001 CAggATCAAg TgTTCAggAg CgTACTgATT ggACATTTCC AAAgCCTgCT
2051 CgTAggTTgC AACCgATAgg gTTgTAgAgT gTgCAATACA CTTgCgTACA
2101 ATTTCAACCC TTggCAACTg CACAgCTTgg TTgTgAACAg CATCTTCAAT
2151 TCTggCAAgC TCCTTgTCTg TCATATCgAC AgCCAACAgA ATCACCTggg
2201 AATCAATACC ATgTTCAgCT TgAgCAgAAg gTCTgAggCA ACgAAATCTg
2251 gATCAgCgTA TTTATCAgCA ATAACTAgAA CTTCAgAAgg CCCAgCAggC
2301 ATgTCAATAC TACACAgggC TgATgTgTCA TTTTgAACCA TCATCTTggC
2351 AgCAgTAACg AACTggTTTC CTggACCAAA TATTTTgTCA CACTTAggAA
2401 CAgTTTCTgT TCCgTAAgCC ATAgCAgCTA CTgCCTgggC gCCTCCTgCT
2451 AgCACgATAC ACTTAgCACC AACCTTgTgg gCAACgTAgA TgACTTCTgg
2501 ggTAAgggTA CCATCCTTCT TAggTggAgA TgCAAAAACA ATTTCTTTgC
2551 AACCAgCAAC TTTggCAggA ACACCCAgCA TCAgggAAgT ggAAggCAgA
2601 ATTgCggTTC CACCAggAAT ATAgAggCCA ACTTTCTCAA TAggTCTTgC
2651 AAAACgAgAg CAgACTACAC CAgggCAAgT CTCAACTTgC AACgTCTCCg
2701 TTAgTTgAgC TTCATggAAT TTCCTgACgT TATCTATAgA gAgATCAATg

2751 gCTCTCTTAA CgTTATCTgg CAATTgCATA AgTTCCTCTg ggAAAggAgC
2801 TTCTAACACA ggTgTCTTCA AAgCgACTCC ATCAAACTTg gCAgTTAgTT
2851 CTAAAAgggC TTTgTCACCA TTTTgACgAA CATTgTCgAC AATTggTTTg
2901 ACTAATTCCA TAATCTgTTC CgTTTTCTgg ATAggACgAC gAAgggCATC
2951 TTCAATTTCT TgTgAggAgg CCTTAgAAAC gTCAATTTTg CACAATTCAA
3001 TACgACCTTC AgAAgggACT TCTTTAggTT TggATTCTTC TTTAggTTgT
3051 TCCTTggTgT ATCCTggCTT ggCATCTCCT TTCCTTCTAg TgACCTTTAg
3101 ggACTTCATA TCCAggTTTC TCTCCACCTC gTCCAACgTC ACACCgTACT
3151 TggCACATCT AACTAATgCA AAATAAAATA AgTCAgCACA TTCCCAggCT
3201 ATATCTTCCT TggATTTAgC TTCTgCAAgT TCATCAgCTT CCTCCCTAAT
3251 TTTAgCgTTC AACAAAACTT CgTCgTCAAA TAACCgTTTg gTATAAgAAC
3301 CTTCTggAgC ATTgCTCTTA CgATCCCACA AggTgCTTCC ATggCTCTAA
3351 gACCCTTTgA TTggCCAAAA CAggAAgTgC gTTCCAAgTg ACAgAAACCA
3401 ACACCTgTTT gTTCAACCAC AAATTTCAAg CAgTCTCCAT CACAATCCAA

Table ~~250~~ 23, continued

3451 TTCgATACCC AgCAACTTTT gAgTTCgTCC AgATgTAgCA CCTTTATACC
3501 ACAAACCgTg ACgACgAgAT TggTAgACTC CAgTTTgTgT CCTTATAgCC
3551 TCCggAATAg ACTTTTTggA CgAgTACACC AggCCCAACg AgTAATTAgA
3601 AgAgTCAgCC ACCAAAgTAg TgAATAgACC ATCggggCgg TCAgTAgTCA
3651 AAgACgCCAA CAAAATTTCA CTgACAgggA ACTTTTTgAC ATCTTCAgAA
3701 AgTTCgTATT CAgTAgTCAA TTgCCgAgCA TCAATAATgg ggATTATACC
3751 AgAAgCAACA gTggAAgTCA CATCTACCAA CTTTgCggTC TCAgAAAAAg
3801 CATAAACAgT TCTACTACCg CCATTAgTgA AACTTTTCAA ATCgCCCAgT
3851 ggAgAAgAAA AAggCACAgC gATACTAgCA TTAgCgggCA AggATgCAAC
3901 TTTATCAACC AgggTCCTAT AgATAACCCT AgCgCCTggg ATCATCCTTT
3951 ggACAACTCT TTCTgCCAAA TCTAggTCCA AAATCACTTC ATTgATACCA
4001 TTATACggAT gACTCAACTT gCACATTAAC TTgAAgCTCA gTCgATTgAg
4051 TgAACTTgAT CAggTTgTgC AgCTggTCAg CAgCATAggg AAACACggCT
4101 TTTCCTACCA AACTCAAggA ATTATCAAAC TCTgCAACAC TTgCgTATgC
4151 AggTAgCAAg ggAAATgTCA TACTTgAAgT CggACAgTgA gTgTAgTCTT
4201 gAgAAATTCT gAAgCCgTAT TTTTATTATC AgTgAgTCAg TCATCAggAg
4251 ATCCTCTACg CCggACgCAT CgTggCCggC ATCACCggCg CCACAggTgC
4301 ggTTgCTggC gCCTATATCg CCgACATCAC CgATggggAA gATCgggCTC

4351 gCCACTTCgg gCTCATgAgC gCTTgTTTCg gCgTgggTAT ggTggCAggC
4401 CCCgTggCCg ggggACTgTT gggCgCCATC TCCTTgCATg CACCATTCCT
4451 TgCggCggCg gTgCTCAACg gCCTCAACCT ACTACTgggC TgCTTCCTAA
4501 TgCAggAgTC gCATAAgggA gAgCgTCgAg TATCTATgAT TggAAgTATg
4551 ggAATggTgA TACCCgCATT CTTCAgTgTC TTgAggTCTC CTATCAgATT
4601 ATgCCCAACT AAAgCAACCg gAggAggAgA TTTCATggTA AATTTCTCTg
4651 ACTTTTggTC ATCAgTAgAC TCgAACTgTg AgACTATCTC ggTTATgACA
4701 gCAgAAATgT CCTTCTTggA gACAgTAAAT gAAgTCCCAC CAATAAAgAA
4751 ATCCTTgTTA TCAggAACAA ACTTCTTgTT TCgAACTTTT TCggTgCCTT
4801 gAACTATAAA ATgTAgAgTg gATATgTCgg gTAggAATgg AgCgggCAAA
4851 TgCTTACCTT CTggACCTTC AAgAggTATg TAgggTTTgT AgATACTgAT
4901 gCCAACTTCA gTgACAACgT TgCTATTTCg TTCAAACCAT TCCgAATCCA
4951 gAgAAATCAA AgTTgTTTgT CTACTATTgA TCCAAgCCAg TgCggTCTTg
5001 AAACTgACAA TAgTgTgCTC gTgTTTTgAg gTCATCTTTg TATgAATAAA
5051 TCTAgTCTTT gATCTAAATA ATCTTgACgA gCCAAggCgA TAAATACCCA
5101 AATCTAAAAC TCTTTTAAAA CgTTAAAAgg ACAAgTATgT CTgCCTgTAT
5151 TAAACCCCAA ATCAgCTCgT AgTCTgATCC TCATCAACTT gAggggCACT
5201 ATCTTgTTTT AgAgAAATTT gCggAgATgC gATATCgAgA AAAAggTACg
5251 CTgATTTTAA ACgTgAAATT TATCTCAAgA TCgCggCCgC gATCTCgAAT
5301 AATAACTgTT ATTTTTCAgT gTTCCCgATC TgCgTCTATT TCACAATACC

Table ~~250~~ 23, continued

5351 AACATgAgTC AgCTTATCgA TgATAAgCTg TCAAACATgA gAATTAATTC
5401 gATgATAAgC TgTCAAACAT gAgAAATCTT gAAgACgAAA gggCCTCgTg
5451 ATACgCCTAT TTTTATAggT TAATgTCATg ATAATAATgg TTTCTTAgAC
5501 gTCAggTggC ACTTTTCggg gAAATgTgCg CggAACCCCT ATTTgTTTAT
5551 TTTTCTAAAT ACATTCAAAT ATgTATCCgC TCATgAgACA ATAACCCTgA
5601 TAAATgCTTC AATAATATTg AAAAAggAAg AgTATgAgTA TTCAACATTT
5651 CCgTgTCgCC CTTATTCCCT TTTTTgCggC ATTTTgCCTT CCTgTTTTTg
5701 CTCACCCAgA AACgCTggTg AAAgTAAAAg ATgCTgAAgA TCAgTTgggT
5751 gCACgAgTgg gTTACATCgA ACTggATCTC AACAgCggTA AgATCCTTgA
5801 gAgTTTTCgC CCCgAAgAAC gTTTTCCAAT gATgAgCACT TTTAAAgTTC
5851 TgCTATgTgg CgCggTATTA TCCCgTgTTg ACgCCgggCA AgAgCAACTC
5901 ggTCgCCgCA TACACTATTC TCAgAATgAC TTggTTgAgT ACTCACCAgT

5951 CACAgAAAAg CATCTTACgg ATggCATgAC AgTAAgAgAA TTATgCAgTg
6001 CTgCCATAAC CATgAgTgAT AACACTgCgg CCAACTTACT TCTgACAACg
6051 ATCggAggAC CgAAggAgCT AACCgCTTTT TTgCACAACA TgggggATCA
6101 TgTAACTCgC CTTgATCgTT gggAACCggA gCTgAATgAA gCCATACCAA
6151 ACgACgAgCg TgACACCACg ATgCCTgCAg CAATggCAAC AACgTTgCgC
6201 AAACTATTAA CTggCgAACT ACTTACTCTA gCTTCCCggC AACAATTAAT
6251 AgACTggATg gAggCggATA AAgTTgCAgg ACCACTTCTg CgCTCggCCC
6301 TTCCggCTgg CTggTTTATT gCTgATAAAT CTggAgCCgg TgAgCgTggg
6351 TCTCgCggTA TCATTgCAgC ACTggggCCA gATggTAAgC CCTCCCgTAT
6401 CgTAgTTATC TACACgACgg ggAgTCAggC AACTATggAT gAACgAAATA
6451 gACAgATCgC TgAgATAggT gCCTCACTgA TTAAgCATTg gTAACTgTCA
6501 gACCAAgTTT ACTCATATAT ACTTTAgATT gATTTAAATT gTAAACgTTA
6551 ATATTTTgTT AAAATTCgCg TTAAATTTTT gTTAAATCAg CTCATTTTTT
6601 AACCAATAgg CCgAAATCgg CAAAATCCCT TATAAATCAA AAgAATAgAC
6651 CgAgATAggg TTgAgTgTTg TTCCAgTTTg gAACAAgAgT CCACTATTAA
6701 AgAACgTggA CTCCAACgTC AAAgggCgAA AAACCgTCTA TCAgggCgAT
6751 ggCCCACTAC gTgAACCATC ACCCTAATCA AgTTTTTTgg ggTCgAggTg
6801 CCgTAAAgCA CTAAATCggA ACCCTAAAgg gAgCCCCCgA TTTAgAgCTT
6851 gACggggAAA gCCggCgAAC gTggCgAgAA AggAAgggAA gAAAgCgAAA
6901 ggAgCgggCg CTAgggCgCT ggCAAgTgTA gCggTCACgC TgCgCgTAAC
6951 CACCACACCC gCCgCgCTTA ATgCgCCgCT ACAgggCgCg TAAAAggATC
7001 TAggTgAAgA TCCTTTTTgA TAATCTCATg ACCAAAATCC CTTAACgTgA
7051 gTTTTCgTTC CACTgAgCgT CAgACCCCgT AgAAAAgATC AAAggATCTT
7101 CTTgAgATCC TTTTTTTCTg CgCgTAATCT gCTgCTTgCA AACAAAAAAA
7151 CCACCgCTAC CAgCggTggT TTgTTTgCCg gATCAAgAgC TACCAACTCT
7201 TTTTCCgAAg gTAACTggCT TCAgCAgAgC gCAgATACCA AATACTgTCC

Table ~~250~~ 23, continued

7251 TTCTAgTgTA gCCgTAgTTA ggCCACCACT TCAAgAACTC TgTAgCACCg
7301 CCTACATACC TCgCTCTgCT AATCCTgTTA CCAgTggCTg CTgCCAgTgg
7351 CgATAAgTCg TgTCTTACCg ggTTggACTC AAgACgATAg TTACCggATA
7401 AggCgCAgCg gTCgggCTgA ACgggggggTT CgTgCACACA gCCCAgCTTg
7451 gAgCgAACgA CCTACACCgA ACTgAgATAC CTACAgCgTg AgCATTgAgA
7501 AAgCgCCACg CTTCCCgAAg ggAgAAAggC ggACAggTAT CCggTAAgCg
7551 gCAgggTCgg AACAggAgAg CgCACgAggg AgCTTCCAgg gggAAACgCC
7601 TggTATCTTT ATAgTCCTgT CgggTTTCgC CACCTCTgAC TTgAgCgTCg
7651 ATTTTTgTgA TgCTCgTCAg gggggCggAg CCTATggAAA AACgCCAgCA
7701 ACgCggCCTT TTTACggTTC CTggCCTTTT gCTggCCTTT TgCTCACATg
7751 TTCTTTCCTg CgTTATCCCC TgATTCTgTg gATAACCgTA TTACCgCCTT
7801 TgAgTgAgCT gATACCgCTC gCCgCAgCCg AACgACCgAg CgCAgCgAgT
7851 CAgTgAgCgA ggAAgCggAA gAgCgCCTgA TgCggTATTT TCTCCTTACg
7901 CATCTgTgCg gTATTTCACA CCgCATATgg TgCACTCTCA gTACAATCTg
7951 CTCTgATgCC gCATAgTTAA gCCAgTATAC ACTCCgCTAT CgCTACgTgA
8001 CTgggTCATg gCTgCgCCCC gACACCCgCC AACACCCgCT gACgCgCCCT
8051 gACgggCTTg TCTgCTCCCg gCATCCgCTT ACAgACAAgC TgTgACCgTC
8101 TCCgggAgCT gCATgTgTCA gAggTTTTCA CCgTCATCAC CgAAACgCgC
8151 gAggCAg

Please replace Table 251 beginning on page 95 to page 101 with the following amended Table:

Table ~~251~~ 24: pHIL-D2(MFαPrePro::EPI-HNE-3) 8584 b.p.

DNA has SEQ ID NO. 071; Encoded polypeptide has SEQ ID NO. 072. DNA is circular and double stranded, only one strand is shown. Translation of the protein to be expressed is shown.

```
                1          2          3          4          5
          1234567890 1234567890 1234567890 1234567890 1234567890
        1 AgATCgCggC CgCgATCTAA CATCCAAAgA CgAAAggTTg AATgAAACCT
       51 TTTTgCCATC CgACATCCAC AggTCCATTC TCACACATAA gTgCCAAACg
      101 CAACAggAgg ggATACACTA gCAgCAgACC gTTgCAAACg CAggACCTCC
      151 ACTCCTCTTC TCCTCAACAC CCACTTTTgC CATCgAAAAA CCAgCCCAgT
      201 TATTgggCTT gATTggAgCT CgCTCATTCC AATTCCTTCT ATTAggCTAC
      251 TAACACCATg ACTTTATTAg CCTgTCTATC CTggCCCCCC TggCgAggTC
      301 ATgTTTgTTT ATTTCCgAAT gCAACAAgCT CCgCATTACA CCCgAACATC
      351 ACTCCAgATg AgggCTTTCT gAgTgTgggg TCAAATAgTT TCATgTTCCC
      401 AAATggCCCA AAACTgACAg TTTAAACgCT gTCTTggAAC CTAATATgAC
      451 AAAAgCgTgA TCTCATCCAA gATgAACTAA gTTTggTTCg TTgAAATgCT
      501 AACggCCAgT TggTCAAAAA gAAACTTCCA AAAgTCgCCA TACCgTTTgT
      551 CTTgTTTggT ATTgATTgAC gAATgCTCAA AAATAATCTC ATTAATgCTT
      601 AgCgCAgTCT CTCTATCgCT TCTgAACCCg gTggCACCTg TgCCgAAACg
      651 CAAATggggA AACAACCCgC TTTTTggATg ATTATgCATT gTCCTCCACA
      701 TTgTATgCTT CCAAgATTCT ggTgggAATA CTgCTgATAg CCTAACgTTC
      751 ATgATCAAAA TTTAACTgTT CTAACCCCTA CTTgACAggC AATATATAAA
      801 CAgAAggAAg CTgCCCTgTC TTAAACCTTT TTTTTTATCA TCATTATTAg
      851 CTTACTTTCA TAATTgCgAC TggTTCCAAT TgACAAgCTT TTgATTTTAA
      901 CgACTTTTAA CgACAACTTg AgAAgATCAA AAAACAACTA ATTATTCgAA
!                                                      BstBI
    ACg
!     M   R   F   P   S   I   F   T   A   V   L   F   A
13
     ATg AgA TTC CCA TCT ATC TTC ACT gCT gTT TTg TTC gCT
!         |  BsaBI    |
```

```
!
!  A    S    S    A    L    A    A    P    V    N    T    T    T    E
27
   gCT TCC TCT gCT TTg gCT g**CT CCA** gTT AAC ACC ACT ACT gAA
!                                 BpmI   HpaI                  BbsI
!
!  D    E    T    A    Q    I    P    A    E    A    V    I    G    Y
41
   gAC gAg ACT gCT CAA ATT CCT gCT gAg gCT gTC ATC ggT TAC
!BbsI
!
!  S    D    L    E    G    D    F    D    V    A    V    L    P    F
55
   TCT gAC TTg gAA ggT gAC TTC gAC gTC gCT gTT TTg CCA TTC
!                              AatII
!
!  S    N    S    T    N    N    G    L    L    F    I    N    T    T
69
   TCT AAC TCT ACT AAC AAC ggT TTg TTg TTC ATC AAC ACT ACC
!
!  I    A    S    I    A    A    K    E    E    G    V    S    L    D
83
   ATC gCT TCT ATC gCT gCT AAg gAg gAA ggT gTT TCC TTg gAC
!
!  K    R         A    A    C    N    L    P
91
   AAg AgA     gCT gCT TgT AAC TTg CCA
                 └──── Site of cleavage
!
!  I    V    R    G    P    C    I    A    F    F    P    R    W    A
105
   ATC gTC AgA ggT CCA TgC ATT gCT TTC TTC CCA AgA Tgg gCT
!                      NsiI
!
!  F    D    A    V    K    G    K    C    V    L    F    P    Y    G
119
   TTC gAC gCT gTT AAg ggT AAg TgC gTC TTg TTC CCA TAC ggT
!                                          |  PflMI
!
!  G    C    Q    G    N    G    N    K    F    Y    S    E    K    E
133
   ggT TgT CAA ggT AAC ggT AAC AAg TTC TAC TCT gAg AAg gAg
! PflMI
!
!  C    R    E    Y    C    G    V    P    .    .
141
   TgT AgA gAg TAC TgT ggT gTT CCA TAg TAA gAATTCgCCT
!                                        EcoRI

                                                  TAgACATg
```

1401 ACTgTTCCTC AgTTCAAgTT gggCATTACg AgAAgACCgg TCTTgCTAgA
1451 TTCTAATCAA gAggATgTCA gAATgCCATT TgCCTgAgAg ATgCAggCTT
1501 CATTTTTgAT ACTTTTTTAT TTgTAACCTA TATAgTATAg gATTTTTTTT
1551 gTCATTTTgT TTCTTCTCgT ACgAgCTTgC TCCTgATCAg CCTATCTCgC
1601 AgCTgATgAA TATCTTgTgg TAggggTTTg ggAAAATCAT TCgAgTTTgA
1651 TgTTTTTCTT ggTATTTCCC ACTCCTCTTC AgAgTACAgA AgATTAAgTg
1701 AgAAgTTCgT TTgTgCAAgC TTATCgATAA gCTTTAATgC ggTAgTTTAT
1751 CACAgTTAAA TTgCTAACgC AgTCAggCAC CgTgTATgAA ATCTAACAAT
1801 gCgCTCATCg TCATCCTCgg CACCgTCACC CTggATgCTg TAggCATAgg
1851 CTTggTTATg CCggTACTgC CgggCCTCTT gCgggATATC gTCCATTCCg

Table ~~251~~ 24, continued

1901 ACAgCATCgC CAgTCACTAT ggCgTgCTgC TAgCgCTATA TgCgTTgATg
1951 CAATTTCTAT gCgCACCCgT TCTCggAgCA CTgTCCgACC gCTTTggCCg
2001 CCgCCCAgTC CTgCTCgCTT CgCTACTTgg AgCCACTATC gACTACgCgA
2051 TCATggCgAC CACACCCgTC CTgTggATCT ATCgAATCTA AATgTAAgTT
2101 AAAATCTCTA AATAATTAAA TAAgTCCCAg TTTCTCCATA CgAACCTTAA
2151 CAgCATTgCg gTgAgCATCT AgACCTTCAA CAgCAgCCAg ATCCATCACT
2201 gCTTggCCAA TATgTTTCAg TCCCTCAggA gTTACgTCTT gTgAAgTgAT
2251 gAACTTCTgg AAggTTgCAg TgTTAACTCC gCTgTATTgA CgggCATATC
2301 CgTACgTTgg CAAAgTgTgg TTggTACCgg AggAgTAATC TCCACAACTC
2351 TCTggAgAgT AggCACCAAC AAACACAgAT CCAgCgTgTT gTACTTgATC
2401 AACATAAgAA gAAgCATTCT CgATTTgCAg gATCAAgTgT TCAggAgCgT
2451 ACTgATTggA CATTTCCAAA gCCTgCTCgT AggTTgCAAC CgATAgggTT
2501 gTAgAgTgTg CAATACACTT gCgTACAATT TCAACCCTTg gCAACTgCAC
2551 AgCTTggTTg TgAACAgCAT CTTCAATTCT ggCAAgCTCC TTgTCTgTCA
2601 TATCgACAgC CAACAgAATC ACCTgggAAT CAATACCATg TTCAgCTTgA
2651 gCAgAAggTC TgAggCAACg AAATCTggAT CAgCgTATTT ATCAgCAATA
2701 ACTAgAACTT CAgAAggCCC AgCAggCATg TCAATACTAC ACAgggCTgA
2751 TgTgTCATTT TgAACCATCA TCTTggCAgC AgTAACgAAC TggTTTCCTg
2801 gACCAAATAT TTTgTCACAC TTAggAACAg TTTCTgTTCC gTAAgCCATA
2851 gCAgCTACTg CCTgggCgCC TCCTgCTAgC ACgATACACT TAgCACCAAC
2901 CTTgTgggCA ACgTAgATgA CTTCTggggT AAgggTACCA TCCTTCTTAg
2951 gTggAgATgC AAAAACAATT TCTTTgCAAC CAgCAACTTT ggCAggAACA

3001 CCCAgCATCA gggAAgTggA AggCAgAATT gCggTTCCAC CAggAATATA
3051 gAggCCAACT TTCTCAATAg gTCTTgCAAA ACgAgAgCAg ACTACACCAg
3101 ggCAAgTCTC AACTTgCAAC gTCTCCgTTA gTTgAgCTTC ATggAATTTC
3151 CTgACgTTAT CTATAgAgAg ATCAATggCT CTCTTAACgT TATCTggCAA
3201 TTgCATAAgT TCCTCTgggA AAggAgCTTC TAACACAggT gTCTTCAAAg
3251 CgACTCCATC AAACTTggCA gTTAgTTCTA AAAgggCTTT gTCACCATTT
3301 TgACgAACAT TgTCgACAAT TggTTTgACT AATTCCATAA TCTgTTCCgT
3351 TTTCTggATA ggACgACgAA gggCATCTTC AATTTCTTgT gAggAggCCT
3401 TAgAAACgTC AATTTTgCAC AATTCAATAC gACCTTCAgA AgggACTTCT
3451 TTAggTTTgg ATTCTTCTTT AggTTgTTCC TTggTgTATC CTggCTTggC
3501 ATCTCCTTTC CTTCTAgTgA CCTTTAgggA CTTCATATCC AggTTTCTCT
3551 CCACCTCgTC CAACgTCACA CCgTACTTgg CACATCTAAC TAATgCAAAA
3601 TAAAATAAgT CAgCACATTC CCAggCTATA TCTTCCTTgg ATTTAgCTTC
3651 TgCAAgTTCA TCAgCTTCCT CCCTAATTTT AgCgTTCAAC AAAACTTCgT
3701 CgTCAAATAA CCgTTTggTA TAAgAACCTT CTggAgCATT gCTCTTACgA
3751 TCCCACAAgg TgCTTCCATg gCTCTAAgAC CCTTTgATTg gCCAAAACAg

Table ~~251~~ 24, continued

3801 gAAgTgCgTT CCAAgTgACA gAAACCAACA CCTgTTTgTT CAACCACAAA
3851 TTTCAAgCAg TCTCCATCAC AATCCAATTC gATACCCAgC AACTTTTgAg
3901 TTCgTCCAgA TgTAgCACCT TTATACCACA AACCgTgACg ACgAgATTgg
3951 TAgACTCCAg TTTgTgTCCT TATAgCCTCC ggAATAgACT TTTTggACgA
4001 gTACACCAgg CCCAACgAgT AATTAgAAgA gTCAgCCACC AAAgTAgTgA
4051 ATAgACCATC ggggCggTCA gTAgTCAAAg ACgCCAACAA AATTTCACTg
4101 ACAgggAACT TTTTgACATC TTCAgAAAgT TCgTATTCAg TAgTCAATTg
4151 CCgAgCATCA ATAATggggA TTATACCAgA AgCAACAgTg gAAgTCACAT
4201 CTACCAACTT TgCggTCTCA gAAAAAgCAT AAACAgTTCT ACTACCgCCA
4251 TTAgTgAAAC TTTTCAAATC gCCCAgTggA gAAgAAAAAg gCACAgCgAT
4301 ACTAgCATTA gCgggCAAgg ATgCAACTTT ATCAACCAgg gTCCTATAgA
4351 TAACCCTAgC gCCTgggATC ATCCTTTggA CAACTCTTTC TgCCAAATCT
4401 AggTCCAAAA TCACTTCATT gATACCATTA TACggATgAC TCAACTTgCA
4451 CATTAACTTg AAgCTCAgTC gATTgAgTgA ACTTgATCAg gTTgTgCAgC
4501 TggTCAgCAg CATAgggAAA CACggCTTTT CCTACCAAAC TCAAggAATT
4551 ATCAAACTCT gCAACACTTg CgTATgCAgg TAgCAAgggA AATgTCATAC

4601 TTgAAgTCgg ACAgTgAgTg TAgTCTTgAg AAATTCTgAA gCCgTATTTT
4651 TATTATCAgT gAgTCAgTCA TCAggAgATC CTCTACgCCg gACgCATCgT
4701 ggCCggCATC ACCggCgCCA CAggTgCggT TgCTggCgCC TATATCgCCg
4751 ACATCACCgA TggggAAgAT CgggCTCgCC ACTTCgggCT CATgAgCgCT
4801 TgTTTCggCg TgggTATggT ggCAggCCCC gTggCCgggg gACTgTTggg
4851 CgCCATCTCC TTgCATgCAC CATTCCTTgC ggCggCggTg CTCAACggCC
4901 TCAACCTACT ACTgggCTgC TTCCTAATgC AggAgTCgCA TAAgggAgAg
4951 CgTCgAgTAT CTATgATTgg AAgTATgggA ATggTgATAC CCgCATTCTT
5001 CAgTgTCTTg AggTCTCCTA TCAgATTATg CCCAACTAAA gCAACCggAg
5051 gAggAgATTT CATggTAAAT TTCTCTgACT TTTggTCATC AgTAgACTCg
5101 AACTgTgAgA CTATCTCggT TATgACAgCA gAAATgTCCT TCTTggAgAC
5151 AgTAAATgAA gTCCCACCAA TAAAgAAATC CTTgTTATCA ggAACAAACT
5201 TCTTgTTTCg AACTTTTTCg gTgCCTTgAA CTATAAAATg TAgAgTggAT

*Bst*BI

5251 ATgTCgggTA ggAATggAgC gggCAAATgC TTACCTTCTg gACCTTCAAg
5301 AggTATgTAg ggTTTgTAgA TACTgATgCC AACTTCAgTg ACAACgTTgC
5351 TATTTCgTTC AAACCATTCC gAATCCAgAg AAATCAAAgT TgTTTgTCTA
5401 CTATTgATCC AAgCCAgTgC ggTCTTgAAA CTgACAATAg TgTgCTCgTg
5451 TTTTgAggTC ATCTTTgTAT gAATAAATCT AgTCTTTgAT CTAAATAATC
5501 TTgACgAgCC AAggCgATAA ATACCCAAAT CTAAAACTCT TTTAAAACgT
5551 TAAAAggACA AgTATgTCTg CCTgTATTAA ACCCCAAATC AgCTCgTAgT

Table ~~251~~ 24, continued

5601 CTgATCCTCA TCAACTTgAg gggCACTATC TTgTTTTAgA gAAATTTgCg
5651 gAgATgCgAT ATCgAgAAAA AggTACgCTg ATTTTAAACg TgAAATTTAT
5701 CTCAAgATCg CggCCgCgAT CTCgAATAAT AACTgTTATT TTTCAgTgTT
5751 CCCgATCTgC gTCTATTTCA CAATACCAAC ATgAgTCAgC TTATCgATgA
5801 TAAgCTgTCA AACATgAgAA TTAATTCgAT gATAAgCTgT CAAACATgAg
5851 AAATCTTgAA gACgAAAggg CCTCgTgATA CgCCTATTTT TATAggTTAA
5901 TgTCATgATA ATAATggTTT CTTAgACgTC AggTggCACT TTTCggggAA

*Aat*II

5951 ATgTgCgCgg AACCCCTATT TgTTTATTTT TCTAAATACA TTCAAATATg
6001 TATCCgCTCA TgAgACAATA ACCCTgATAA ATgCTTCAAT AATATTgAAA
6051 AAggAAgAgT ATgAgTATTC AACATTTCCg TgTCgCCCTT ATTCCCTTTT

6101 TTgCggCATT TTgCCTTCCT gTTTTTgCTC ACCCAgAAAC gCTggTgAAA
6151 gTAAAAgATg CTgAAgATCA gTTgggTgCA CgAgTgggTT ACATCgAACT
6201 ggATCTCAAC AgCggTAAgA TCCTTgAgAg TTTTCgCCCC gAAgAACgTT
6251 TTCCAATgAT gAgCACTTTT AAAgTTCTgC TATgTggCgC ggTATTATCC
6301 CgTgTTgACg CCgggCAAgA gCAACTCggT CgCCgCATAC ACTATTCTCA
6351 gAATgACTTg gTTgAgTACT CACCAgTCAC AgAAAAgCAT CTTACggATg
6401 gCATgACAgT AAgAgAATTA TgCAgTgCTg CCATAACCAT gAgTgATAAC
6451 ACTgCggCCA ACTTACTTCT gACAACgATC ggAggACCgA AggAgCTAAC
6501 CgCTTTTTTg CACAACATgg gggATCATgT AACTCgCCTT gATCgTTggg
6551 AACCggAgCT gAATgAAgCC ATACCAAACg ACgAgCgTgA CACCACgATg
6601 CCTgCAgCAA TggCAACAAC gTTgCgCAAA CTATTAACTg gCgAACTACT
6651 TACTCTAgCT TCCCggCAAC AATTAATAgA CTggATggAg gCggATAAAg
6701 TTgCAggACC ACTTCTgCgC TCggCCCTTC CggCTggCTg gTTTATTgCT
6751 gATAAATCTg gAgCCggTgA gCgTgggTCT CgCggTATCA TTgCAgCACT
6801 ggggCCAgAT ggTAAgCCCT CCCgTATCgT AgTTATCTAC ACgACggggA
6851 gTCAggCAAC TATggATgAA CgAAATAgAC AgATCgCTgA gATAggTgCC
6901 TCACTgATTA AgCATTggTA ACTgTCAgAC CAAgTTTACT CATATATACT
6951 TTAgATTgAT TTAAATTgTA AACgTTAATA TTTTgTTAAA ATTCgCgTTA
7001 AATTTTTgTT AAATCAgCTC ATTTTTTAAC CAATAggCCg AAATCggCAA
7051 AATCCCTTAT AAATCAAAAg AATAgACCgA gATAgggTTg AgTgTTgTTC
7101 CAgTTTggAA CAAgAgTCCA CTATTAAAgA ACgTggACTC CAACgTCAAA
7151 gggCgAAAAA CCgTCTATCA gggCgATggC CCACTACgTg AACCATCACC
7201 CTAATCAAgT TTTTTggggT CgAggTgCCg TAAAgCACTA AATCggAACC
7251 CTAAAgggAg CCCCCgATTT AgAgCTTgAC ggggAAAgCC ggCgAACgTg
7301 gCgAgAAAgg AAgggAAgAA AgCgAAAggA gCgggCgCTA gggCgCTggC
7351 AAgTgTAgCg gTCACgCTgC gCgTAACCAC CACACCCgCC gCgCTTAATg
7401 CgCCgCTACA gggCgCgTAA AAggATCTAg gTgAAgATCC TTTTTgATAA

Table ~~251~~ 24, continued

7451 TCTCATgACC AAAATCCCTT AACgTgAgTT TTCgTTCCAC TgAgCgTCAg
7501 ACCCCgTAgA AAAgATCAAA ggATCTTCTT gAgATCCTTT TTTTCTgCgC
7551 gTAATCTgCT gCTTgCAAAC AAAAAAACCA CCgCTACCAg CggTggTTTg
7601 TTTgCCggAT CAAgAgCTAC CAACTCTTTT TCCgAAggTA ACTggCTTCA
7651 gCAgAgCgCA gATACCAAAT ACTgTCCTTC TAgTgTAgCC gTAgTTAggC

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7701 CACCACTTCA AgAACTCTgT AgCACCgCCT ACATACCTCg CTCTgCTAAT
7751 CCTgTTACCA gTggCTgCTg CCAgTggCgA TAAgTCgTgT CTTACCgggT
7801 TggACTCAAg ACgATAgTTA CCggATAAgg CgCAgCggTC gggCTgAACg
7851 ggggggTTCgT gCACACAgCC CAgCTTggAg CgAACgACCT ACACCgAACT
7901 gAgATACCTA CAgCgTgAgC ATTgAgAAAg CgCCACgCTT CCCgAAgggA
7951 gAAAggCggA CAggTATCCg gTAAgCggCA gggTCggAAC AggAgAgCgC
8001 ACgAgggAgC TTCCAgggggg AAACgCCTgg TATCTTTATA gTCCTgTCgg
8051 gTTTCgCCAC CTCTgACTTg AgCgTCgATT TTTgTgATgC TCgTCAgggg
8101 ggCggAgCCT ATggAAAAAC gCCAgCAACg CggCCTTTTT ACggTTCCTg
8151 gCCTTTTgCT ggCCTTTTgC TCACATgTTC TTTCCTgCgT TATCCCCTgA
8201 TTCTgTggAT AACCgTATTA CCgCCTTTgA gTgAgCTgAT ACCgCTCgCC
8251 gCAgCCgAAC gACCgAgCgC AgCgAgTCAg TgAgCgAggA AgCggAAgAg
8301 CgCCTgATgC ggTATTTTCT CCTTACgCAT CTgTgCggTA TTTCACACCg
8351 CATATggTgC ACTCTCAgTA CAATCTgCTC TgATgCCgCA TAgTTAAgCC
8401 AgTATACACT CCgCTATCgC TACgTgACTg ggTCATggCT gCgCCCCgAC
8451 ACCCgCCAAC ACCCgCTgAC gCgCCCTgAC gggCTTgTCT gCTCCCggCA
8501 TCCgCTTACA gACAAgCTgT gACCgTCTCC gggAgCTgCA TgTgTCAgAg
8551 gTTTTCACCg TCATCACCgA AACgCgCgAg gCAg
```

Restriction map of pHIL-D2(MFαPrePro::EPI-HNE-3)

Non-cutters

*Afl*II	*Apa*I	*Asc*I	*Ava*I	*Avr*II
*Bam*HI	*Bgl*II	*Bss*HII	*Bst*EII	*Mlu*I
*Nru*I	*Pac*I	*Pml*I	*Rsr*II	*Sac*II
*Sfi*I	*Sna*BI	*Spe*I	*Xho*I	*Xma*I

Cutters, 3 or fewer sites

*Aat*II	2 1098 5925	*Bgl*I	3 284 2717 6724
***Afl*III**	**1 8173**	*Bsa*AI	2 7185 8421
***Age*I**	**1 1436**		
*Alw*NI	3 2828 2852 7759		
*Apa*LI	3 6176 7859 8357		
*Ase*I	3 591 5820 6672		

Table ~~251~~ 24, continued

*Bsg*I	2	2545	4494	
*Bsi*WI	2	1568	2301	
*Bsp*DI	2	1723	5793	
BspEI	**1**	**3978**		
BspMI	**1**	**4576**		
Bst1107I	**1**	**8402**		
*Bst*BI(*Asu*II)	2	945	5207	
*Bst*XI	3	711	2765	2896
Bsu36I	**1**	**2223**		
*Dra*III	2	3754	7182	
*Eag*I	3	7	5711	8591
*Eam*1105I	2	5077	6843	
Ecl136I	**1**	**216**		
*Eco*47III	2	1932	4795	
*Eco*NI	3	3433	4923	5293
EcoRI	**1**	**1383**		
*Eco*RV	2	1885	5658	
*Esp*3I(*Bsa*I)	2	3120	8524	
EspI(Bpu1102I)	**1**	**597**		
*Fsp*I	2	1960	6623	
*Hin*dIII	3	885	1717	1729
*Hpa*I	2	1017	2272	
*Kpn*I	2	2323	2934	
*Msc*I	2	2204	3789	
NcoI	**1**	**3766**		
NdeI	**1**	**8351**		
*Ngo*MI	2	4702	7288	
*Nhe*I	2	1929	2875	
*Not*I	3	6	5710	8590
*Nsi*I	2	684	1241	
*Pfl*MI	2	196	1302	
PmeI	**1**	**420**		

*Ppu*MI	2	142	4339	
PstI	**1**	**6602**		
PvuI	**1**	**6476**		
*Pvu*II	2	1600	4497	
SacI	**1**	**216**		
SalI	**1**	**3312**		
*Sca*I	2	1360	6365	
SphI	**1**	**4863**		
*Ssp*I	3	2806	6041	6977
StuI	**1**	**3395**		
Tth111I	**1**	**8426**		
XbaI	**1**	**2168**		
XcmI	**1**	**711**		

Please replace Table 252 beginning on page 102 to page 103 with the following amended Table:

Table ~~252~~ 25: *Bst*BI-*Aat*II-*Eco*RI cassette for expression of EPI-HNE-4

DNA has SEQ ID NO. 073; amino-acid sequence has SEQ ID NO. 074

```
!                M   R   F   P   S   I   F   T
  5'TTCgAA ACg ATg AgA TTC CCA TCT ATC TTC ACT
     BstBI          |  BsaBI    |

!            A   V   L   F   A    13
           gCT gTT TTg TTC gCT
!
!  A   S   S   A   L   A   A   P   V   N   T   T   T   E
27
  gCT TCC TCT gCT TTg gCT gCT CCA gTT AAC ACC ACT ACT gAA
!                          BpmI  HpaI              BbsI
!
!  D   E   T   A   Q   I   P   A   E   A   V   I   G   Y
41
  gAC gAg ACT gCT CAA ATT CCT gCT gAg gCT gTC ATC ggT TAC
! BbsI
!
!  S   D   L   E   G   D   F   D   V   A   V   L   P   F
55
  TCT gAC TTg gAA ggT gAC TTC gAC gTC gCT gTT TTg CCA TTC
!                              AatII
!
!  S   N   S   T   N   N   G   L   L   F   I   N   T   T
69
  TCT AAC TCT ACT AAC AAC ggT TTg TTg TTC ATC AAC ACT ACC
!
!  I   A   S   I   A   A   K   E   E   G   V   S   L   D
83
  ATC gCT TCT ATC gCT gCT AAg gAg gAA ggT gTT TCC TTg gAC
!
!  K   R   E   A   C   N   L   P
91
  AAg AgA gAg gCT TgT AAC TTg CCA
!
!  I   V   R   G   P   C   I   A   F   F   P   R   W   A
105
  ATC gTC AgA ggT CCA TgC ATT gCT TTC TTC CCA AgA Tgg gCT
!                      NsiI
!
!  F   D   A   V   K   G   K   C   V   L   F   P   Y   G
```

```
119
  TTC gAC gCT gTT AAg ggT AAg TgC gTC TTg TTC CCA TAC ggT
!                                             |  PflMI
!
!  G   C   Q   G   N   G   N   K   F   Y   S   E   K   E
133
  ggT TgT CAA ggT AAC ggT AAC AAg TTC TAC TCT gAg AAg gAg
! PflMI
!
!  C   R   E   Y   C   G   V   P   .   .
141
  TgT AgA gAg TAC TgT ggT gTT CCA TAg TAA gAATTC
!                                        EcoRI
```

The DNA is a linear fragment that is double stranded *in vivo*, only one strand is shown. The amino acid sequence is that of a disulfide-containing protein that is processed *in vivo*.

Please replace Table 253 beginning on page 104 to page 109 with the following amended Table:

Table ~~253~~ 26: pD2pick(MFαPrePro::EPI-HNE-3), 8590 bp, CIRCULAR dsDNA, one strand shown.

pD2pick(MFαPrePro::EPI-HNE-3) DNA has SEQ ID NO. 075

Encoded protein has SEQ ID NO. 076

```
                1          2          3          4          5
     1234567890 1234567890 1234567890 1234567890 1234567890
   1 AgATCgCggC CgCgATCTAA CATCCAAAgA CgAAAggTTg AATgAAACCT
  51 TTTTgCCATC CgACATCCAC AggTCCATTC TCACACATAA gTgCCAAACg
 101 CAACAggAgg ggATACACTA gCAgCAgACC gTTgCAAACg CAggACCTCC
 151 ACTCCTCTTC TCCTCAACAC CCACTTTTgC CATCgAAAAA CCAgCCCAgT
 201 TATTgggCTT gATTggAgCT CgCTCATTCC AATTCCTTCT ATTAggCTAC
                     SacI
 251 TAACACCATg ACTTTATTAg CCTgTCTATC CTggCCCCCC TggCgAggTC
 301 ATgTTTgTTT ATTTCCgAAT gCAACAAgCT CCgCATTACA CCCgAACATC
 351 ACTCCAgATg AgggCTTTCT gAgTgTgggg TCAAATAgTT TCATgTTCCC
 401 AAATggCCCA AAACTgACAg TTTAAACgCT gTCTTggAAC CTAATATgAC
                              PmeI
 451 AAAAgCgTgA TCTCATCCAA gATgAACTAA gTTTggTTCg TTgAAATgCT
 501 AACggCCAgT TggTCAAAAA gAAACTTCCA AAAgTCgCCA TACCgTTTgT
 551 CTTgTTTggT ATTgATTgAC gAATgCTCAA AAATAATCTC
ATTAATgCTTAgC

EspI
 604    gCAgTCT CTCTATCgCT TCTgAACCCg gTggCACCTg TgCCgAAACg
 651 CAAATggggA AACAACCCgC TTTTTggATg ATTATgCATT gTCCTCCACA
 701 TTgTATgCTT CCAAgATTCT ggTgggAATA CTgCTgATAg CCTAACgTTC
                     XcmI
 751 ATgATCAAAA TTTAACTgTT CTAACCCCTA CTTgACAggC AATATATAAA
 801 CAgAAggAAg CTgCCCTgTC TTAAACCTTT TTTTTTATCA TCATTATTAg
 851 CTTACTTTCA TAATTgCgAC TggTTCCAAT TgACAAgCTT TTgATTTTAA
```

```
901 CgACTTTTAA CgACAACTTg AgAAgATCAA AAAACAACTA ATTATTCgAA
!                                                    BstBI
951 ACg
!
!      M   R   F   P   S   I   F   T   A   V   L   F   A
  954 ATg AgA TTC CCA TCT ATC TTC ACT gCT gTT TTg TTC gCT
!
!      A   S   S   A   L   A   A   P   V   N   T   T   T
```

Table ~~253~~ 26, continued

```
  993 gCT TCC TCT gCT TTg gCT gCT CCA gTT AAC ACC ACT ACT
!
!      E   D   E   T   A   Q   I   P   A   E   A   V   I
 1032 gAA gAC gAg ACT gCT CAA ATT CCT gCT gAg gCT gTC ATC
!
!      G   Y   S   D   L   E   G   D   F   D   V   A   V
 1071 ggT TAC TCT gAC TTg gAA ggT gAC TTC gAC gTC gCT gTT
                                          AatII
!
!      L   P   F   S   N   S   T   N   N   G   L   L   F
 1110 TTg CCA TTC TCT AAC TCT ACT AAC AAC ggT TTg TTg TTC
!
!      I   N   T   T   I   A   S   I   A   A   K   E   E
 1149 ATC AAC ACT ACC ATC gCT TCT ATC gCT gCT AAg gAg gAA
!
!      G   V   S   L   D   K   R   A   A   C   N   L   P
 1188 ggT gTT TCC TTg gAC AAg AgA gCT gCT TgT AAC TTg CCA
!
!      I   V   R   G   P   C   I   A   F   F   P   R   W
 1227 ATC gTC AgA ggT CCA TgC ATT gCT TTC TTC CCA AgA Tgg
!
!      A   F   D   A   V   K   G   K   C   V   L   F   P
 1266 gCT TTC gAC gCT gTT AAg ggT AAg TgC gTC TTg TTC CCA
!
!      Y   G   G   C   Q   G   N   G   N   K   F   Y   S
 1305 TAC ggT ggT TgT CAA ggT AAC ggT AAC AAg TTC TAC TCT
!
!      E   K   E   C   R   E   Y   C   G   V   P   .   .
 1344 gAg AAg gAg TgT AgA gAg TAC TgT ggT gTT CCA TAg TAA
!
 1383 gAATTC                                      gC CTTAgACATg
!      EcoRI
1401 ACTgTTCCTC AgTTCAAgTT gggCATTACg AgAAgACCgg TCTTgCTAgA
                                                  AegI
1451 TTCTAATCAA gAggATgTCA gAATgCCATT TgCCTgAgAg ATgCAggCTT
```

1501 CATTTTTgAT ACTTTTTTAT TTgTAACCTA TATAgTATAg gATTTTTTTT
1551 gTCATTTTgT TTCTTCTCgT ACgAgCTTgC TCCTgATCAg CCTATCTCgC
1601 AgCTgATgAA TATCTTgTgg TAggggTTTg ggAAAATCAT TCgAgTTTgA
1651 TgTTTTTCTT ggTATTTCCC ACTCCTCTTC AgAgTACAgA AgATTAAgTg
1701 AgAAgTTCgT TTgTgCAAgC TTATCgATAA gCTTTAATgC ggTAgTTTAT
1751 CACAgTTAAA TTgCTAACgC AgTCAggCAC CgTgTATgAA ATCTAACAAT
1801 gCgCTCATCg TCATCCTCgg CACCgTCACC CTggATgCTg TAggCATAgg
1851 CTTggTTATg CCggTACTgC CgggCCTCTT gCgggATATC gTCCATTCCg
1901 ACAgCATCgC CAgTCACTAT ggCgTgCTgC TAgCgCTATA TgCgTTgATg
1951 CAATTTCTAT gCgCACCCgT TCTCggAgCA CTgTCCgACC gCTTTggCCg
2001 CCgCCCAgTC CTgCTCgCTT CgCTACTTgg AgCCACTATC gACTACgCgA
2051 TCATggCgAC CACACCCgTC CTgTggATCT ATCgAATCTA AATgTAAgTT
2101 AAAATCTCTA AATAATTAAA TAAgTCCCAg TTTCTCCATA CgAACCTTAA

Table ~~253~~ 26, continued

2151 CAgCATTgCg gTgAgCA**TCT AgA**CCTTCAA CAgCAgCCAg ATCCATCACT
*Xba*I
2201 gCTTggCCAA TATgTTTCAg TC**CCTCAgg**A gTTACgTCTT gTgAAgTgAT
*Bsu*36I
2251 gAACTTCTgg AAggTTgCAg TgTTAACTCC gCTgTATTgA CgggCATATC
2301 CgTACgTTgg CAAAgTgTgg TTggTACCgg AggAgTAATC TCCACAACTC
2351 TCTggAgAgT AggCACCAAC AAACACAgAT CCAgCgTgTT gTACTTgATC
2401 AACATAAgAA gAAgCATTCT CgATTTgCAg gATCAAgTgT TCAggAgCgT
2451 ACTgATTggA CATTTCCAAA gCCTgCTCgT AggTTgCAAC CgATAgggTT
2501 gTAgAgTgTg CAATACACTT gCgTACAATT TCAACCCTTg gCAACTgCAC
2551 AgCTTggTTg TgAACAgCAT CTTCAATTCT ggCAAgCTCC TTgTCTgTCA
2601 TATCgACAgC CAACAgAATC ACCTgggAAT CAATACCATg TTCAgCTTgA
2651 gCAgAAggTC TgAggCAACg AAATCTggAT CAgCgTATTT ATCAgCAATA
2701 ACTAgAACTT CAgAAggCCC AgCAggCATg TCAATACTAC ACAgggCTgA
2751 TgTgTCATTT TgAACCATCA TCTTggCAgC AgTAACgAAC TggTTTCCTg
2801 gACCAAATAT TTTgTCACAC TTAggAACAg TTTCTgTTCC gTAAgCCATA
2851 gCAgCTACTg CCTgggCgCC TCCTgCTAgC ACgATACACT TAgCACCAAC
2901 CTTgTgggCA ACgTAgATgA CTTCTggggT AAgggTACCA TCCTTCTTAg

2951 gTggAgATgC AAAAACAATT TCTTTgCAAC CAgCAACTTT ggCAggAACA
3001 CCCAgCATCA gggAAgTggA AggCAgAATT gCggTTCCAC CAggAATATA
3051 gAggCCAACT TTCTCAATAg gTCTTgCAAA ACgAgAgCAg ACTACACCAg
3101 ggCAAgTCTC AACTTgCAAC gTCTCCgTTA gTTgAgCTTC ATggAATTTC
3151 CTgACgTTAT CTATAgAgAg ATCAATggCT CTCTTAACgT TATCTggCAA
3201 TTgCATAAgT TCCTCTgggA AAggAgCTTC TAACACAggT gTCTTCAAAg
3251 CgACTCCATC AAACTTggCA gTTAgTTCTA AAAgggCTTT gTCACCATTT
3301 TgACgAACAT TgTCgACAAT TggTTTgACT AATTCCATAA TCTgTTCCgT
3351 TTTCTggATA ggACgACgAA gggCATCTTC AATTTCTTgT gAgg**AggCCT**

*Stu*I

3401 TAgAAACgTC AATTTTgCAC AATTCAATAC gACCTTCAgA AgggACTTCT
3451 TTAggTTTgg ATTCTTCTTT AggTTgTTCC TTggTgTATC CTggCTTggC
3501 ATCTCCTTTC CTTCTAgTgA CCTTTAgggA CTTCATATCC AggTTTCTCT
3551 CCACCTCgTC CAACgTCACA CCgTACTTgg CACATCTAAC TAATgCAAAA
3601 TAAAATAAgT CAgCACATTC CCAggCTATA TCTTCCTTgg ATTTAgCTTC
3651 TgCAAgTTCA TCAgCTTCCT CCCTAATTTT AgCgTTCAAC AAAACTTCgT
3701 CgTCAAATAA CCgTTTggTA TAAgAACCTT CTggAgCATT gCTCTTACgA
3751 TCCCACAAgg TgCTT**CCATg g**CTCTAAgAC CCTTTgATTg gCCAAAACAg

*Nco*I

Table ~~253~~ 26, continued

3801 gAAgTgCgTT CCAAgTgACA gAAACCAACA CCTgTTTgTT CAACCACAAA
3851 TTTCAAgCAg TCTCCATCAC AATCCAATTC gATACCCAgC AACTTTTgAg
3901 TTCgTCCAgA TgTAgCACCT TTATACCACA AACCgTgACg ACgAgATTgg
3951 TAgACTCCAg TTTgTgTCCT TATAgCC**TCC ggA**ATAgACT TTTTggACgA

*Bsp*EI

4001 gTACACCAgg CCCAACgAgT AATTAgAAgA gTCAgCCACC AAAgTAgTgA
4051 ATAgACCATC ggggCggTCA gTAgTCAAAg ACgCCAACAA AATTTCACTg
4101 ACAgggAACT TTTTgACATC TTCAgAAAgT TCgTATTCAg TAgTCAATTg
4151 CCgAgCATCA ATAATggggA TTATACCAgA AgCAACAgTg gAAgTCACAT
4201 CTACCAACTT TgCggTCTCA gAAAAAgCAT AAACAgTTCT ACTACCgCCA
4251 TTAgTgAAAC TTTTCAAATC gCCCAgTggA gAAgAAAAAg gCACAgCgAT
4301 ACTAgCATTA gCgggCAAgg ATgCAACTTT ATCAACCAgg gTCCTATAgA

4351 TAACCCTAgC gCCTgggATC ATCCTTTggA CAACTCTTTC TgCCAAATCT
4401 AggTCCAAAA TCACTTCATT gATACCATTA TACggATgAC TCAACTTgCA
4451 CATTAACTTg AAgCTCAgTC gATTgAgTgA ACTTgATCAg gTTgTgCAgC
4501 TggTCAgCAg CATAgggAAA CACggCTTTT CCTACCAAAC TCAAggAATT
4551 ATCAAACTCT gCAACACTTg CgTATgCAgg TAgCAAgggA AATgTCATAC
4601 TTgAAgTCgg ACAgTgAgTg TAgTCTTgAg AAATTCTgAA gCCgTATTTT
4651 TATTATCAgT gAgTCAgTCA TCAggAgATC CTCTACgCCg gACgCATCgT
4701 ggCCggCATC ACCggCgCCA CAggTgCggT TgCTggCgCC TATATCgCCg
4751 ACATCACCgA TggggAAgAT CgggCTCgCC ACTTCgggCT CATgAgCgCT
4801 TgTTTCggCg TgggTATggT ggCAggCCCC gTggCCgggg gACTgTTggg
4851 CgCCATCTCC TTgCATgCAC CATTCCTTgC ggCggCggTg CTCAACggCC
4901 TCAACCTACT ACTgggCTgC TTCCTAATgC AggAgTCgCA TAAgggAgAg
4951 CgTCgAgTAT CTATgATTgg AAgTATgggA ATggTgATAC CCgCATTCTT
5001 CAgTgTCTTg AggTCTCCTA TCAgATTATg CCCAACTAAA gCAACCggAg
5051 gAggAgATTT CATggTAAAT TTCTCTgACT TTTggTCATC AgTAgACTCg
5101 AACTgTgAgA CTATCTCggT TATgACAgCA gAAATgTCCT TCTTggAgAC
5151 AgTAAATgAA gTCCCACCAA TAAAgAAATC CTTgTTATCA ggAACAAACT
5201 TCTTgTTTCg CgAACTTTTT CggTgCCTTg AACTATAAAA TgTAgAgTgg
5251 ATATgTCggg TAggAATggA gCgggCAAAT gCTTACCTTC TggACCTTCA
5301 AgAggTATgT AgggTTTgTA gATACTgATg CCAACTTCAg TgACAACgTT
5351 gCTATTTCgT TCAAACCATT CCgAATCCAg AgAAATCAAA gTTgTTTgTC
5401 TACTATTgAT CCAAgCCAgT gCggTCTTgA AACTgACAAT AgTgTgCTCg
5451 TgTTTTgAgg TCATCTTTgT ATgAATAAAT CTAgTCTTTg ATCTAAATAA
5501 TCTTgACgAg CCAAggCgAT AAATACCCAA ATCTAAAACT CTTTTAAAAC
5551 gTTAAAAggA CAAgTATgTC TgCCTgTATT AAACCCCAAA TCAgCTCgTA
5601 gTCTgATCCT CATCAACTTg AggggCACTA TCTTgTTTTA gAgAAATTTg

Table ~~253~~ 26, continued

5651 CggAgATgCg ATATCgAgAA AAAggTACgC TgATTTTAAA CgTgAAATTT
5701 ATCTCAAgAT CgCggCCgCg ATCTCgAATA ATAACTgTTA TTTTTCAgTg
5751 TTCCCgATCT gCgTCTATTT CACAATACCA ACATgAgTCA gCTTATCgAT
5801 gATAAgCTgT CAAACATgAg AATTAATTCg ATgATAAgCT gTCAAACATg
5851 AgAAATCTTg AAgACgAAAg ggCCTCgTgA TACgCCTATT TTTATAggTT

5901 AATgTCATgA TAATAATggT TTCTTAgACg TACgTCAggT ggCACTTTTC
5951 ggggAAATgT gCgCggAACC CCTATTTgTT TATTTTTCTA AATACATTCA
6001 AATATgTATC CgCTCATgAg ACAATAACCC TgATAAATgC TTCAATAATA
6051 TTgAAAAAgg AAgAgTATgA gTATTCAACA TTTCCgTgTC gCCCTTATTC
6101 CCTTTTTTgC ggCATTTTgC CTTCCTgTTT TTgCTCACCC AgAAACgCTg
6151 gTgAAAgTAA AAgATgCTgA AgATCAgTTg ggTgCACgAg TgggTTACAT
6201 CgAACTggAT CTCAACAgCg gTAAgATCCT TgAgAgTTTT CgCCCCgAAg
6251 AACgTTTTCC AATgATgAgC ACTTTTAAAg TTCTgCTATg TggCgCggTA
6301 TTATCCCgTg TTgACgCCgg gCAAgAgCAA CTCggTCgCC gCATACACTA
6351 TTCTCAgAAT gACTTggTTg AgTACTCACC AgTCACAgAA AAgCATCTTA
6401 CggATggCAT gACAgTAAgA gAATTATgCA gTgCTgCCAT AACCATgAgT
6451 gATAACACTg CggCCAACTT ACTTCTgACA ACgATCggAg gACCgAAggA
6501 gCTAACCgCT TTTTTgCACA ACATgggggA TCATgTAACT CgCCTTgATC
6551 gTTgggAACC ggAgCTgAAT gAAgCCATAC CAAACgACgA gCgTgACACC
6601 ACgATgCCTg CAgCAATggC AACAACgTTg CgCAAACTAT TAACTggCgA
6651 ACTACTTACT CTAgCTTCCC ggCAACAATT AATAgACTgg ATggAggCgg
6701 ATAAAgTTgC AggACCACTT CTgCgCTCgg CCCTTCCggC TggCTggTTT
6751 ATTgCTgATA AATCTggAgC CggTgAgCgT gggTCTCgCg gTATCATTgC
6801 AgCACTgggg CCAgATggTA AgCCCTCCCg TATCgTAgTT ATCTACACgA
6851 CggggAgTCA ggCAACTATg gATgAACgAA ATAgACAgAT CgCTgAgATA
6901 ggTgCCTCAC TgATTAAgCA TTggTAACTg TCAgACCAAg TTTACTCATA
6951 TATACTTTAg ATTgATTTAA ATTgTAAACg TTAATATTTT gTTAAAATTC
7001 gCgTTAAATT TTTgTTAAAT CAgCTCATTT TTTAACCAAT AggCCgAAAT
7051 CggCAAAATC CCTTATAAAT CAAAAgAATA gACCgAgATA gggTTgAgTg
7101 TTgTTCCAgT TTggAACAAg AgTCCACTAT TAAAgAACgT ggACTCCAAC
7151 gTCAAAgggC gAAAAACCgT CTATCAgggC gATggCCCAC TACgTgAACC
7201 ATCACCCTAA TCAAgTTTTT TggggTCgAg gTgCCgTAAA gCACTAAATC
7251 ggAACCCTAA AgggAgCCCC CgATTTAgAg CTTgACgggg AAAgCCggCg
7301 AACgTggCgA gAAAggAAgg gAAgAAAgCg AAAggAgCgg gCgCTAgggC
7351 gCTggCAAgT gTAgCggTCA CgCTgCgCgT AACCACCACA CCCgCCgCgC
7401 TTAATgCgCC gCTACAgggC gCgTAAAAgg ATCTAggTgA AgATCCTTTT
7451 TgATAATCTC ATgACCAAAA TCCCTTAACg TgAgTTTTCg TTCCACTgAg
7501 CgTCAgACCC CgTAgAAAAg ATCAAAggAT CTTCTTgAgA TCCTTTTTTT

Table ~~253~~ 26, continued

7551 CTgCgCgTAA TCTgCTgCTT gCAAACAAAA AAACCACCgC TACCAgCggT
7601 ggTTTgTTTg CCggATCAAg AgCTACCAAC TCTTTTTCCg AAggTAACTg
7651 gCTTCAgCAg AgCgCAgATA CCAAATACTg TCCTTCTAgT gTAgCCgTAg
7701 TTAggCCACC ACTTCAAgAA CTCTgTAgCA CCgCCTACAT ACCTCgCTCT
7751 gCTAATCCTg TTACCAgTgg CTgCTgCCAg TggCgATAAg TCgTgTCTTA
7801 CCgggTTggA CTCAAgACgA TAgTTACCgg ATAAggCgCA gCggTCgggC
7851 TgAACgggggg gTTCgTgCAC ACAgCCCAgC TTggAgCgAA CgACCTACAC
7901 CgAACTgAgA TACCTACAgC gTgAgCATTg AgAAAgCgCC ACgCTTCCCg
7951 AAgggAgAAA ggCggACAgg TATCCggTAA gCggCAgggT CggAACAggA
8001 gAgCgCACgA gggAgCTTCC AgggggAAAC gCCTggTATC TTTATAgTCC
8051 TgTCgggTTT CgCCACCTCT gACTTgAgCg TCgATTTTTg TgATgCTCgT
8101 CAgggggggCg gAgCCTATgg AAAAACgCCA gCAACgCggC CTTTTTACgg
8151 TTCCTggCCT TTTgCTggCC TTTTgCTCAC ATgTTCTTTC CTgCgTTATC
8201 CCCTgATTCT gTggATAACC gTATTACCgC CTTTgAgTgA gCTgATACCg
8251 CTCgCCgCAg CCgAACgACC gAgCgCAgCg AgTCAgTgAg CgAggAAgCg
8301 gAAgAgCgCC TgATgCggTA TTTTCTCCTT ACgCATCTgT gCggTATTTC
8351 ACACCgCATA TggTgCACTC TCAgTACAAT CTgCTCTgAT gCCgCATAgT
8401 TAAgCCAgTA TACACTCCgC TATCgCTACg TgACTgggTC ATggCTgCgC
8451 CCCgACACCC gCCAACACCC gCTgACgCgC CCTgACgggC TTgTCTgCTC
8501 CCggCATCCg CTTACAgACA AgCTgTgACC gTCTCCgggA gCTgCATgTg
8551 TCAgAggTTT TCACCgTCAT CACCgAAACg CgCgAggCAg

Please replace Table 254 beginning on page 110 to page 111 with the following amended Table:

Table ~~254~~ 27: restriction map of pD2pick(MFαPrePro::EPI-HNE-3)

Non-cutters				
*Afl*II	*Apa*I	*Asc*I	*Ava*I	*Avr*II
*Bam*HI	*Bgl*II	*Bss*HII	*Bst*EII	*Mlu*I
*Pac*I	*Pml*I	*Rsr*II	*Sac*II	*Sfi*I
*Sna*BI	*Spe*I	*Xho*I	*Xma*I	

Cutters, 3 or fewer sites		
*Aat*II	1	1098
*Afl*III	1	8179
*Age*I	1	1436
*Alw*NI	3	2828 2852 7765
*Apa*LI	3	6182 7865 8363
*Ase*I	3	591 5822 6678
*Bgl*I	3	284 2717 6730
*Bsa*AI	2	7191 8427
*Bsg*I	2	2545 4494
*Bsi*WI	3	1568 2301 5929
*Bsp*DI	2	1723 5795
*Bsp*EI	1	3978
*Bsp*MI	1	4576
*Bst*1107I	1	8408
*Bst*BI(*Asu*II)	1	945
*Bst*XI	3	711 2765 2896
*Bsu*36I	1	2223
*Dra*III	2	3754 7188
*Eag*I	3	7 5713 8597
*Eam*1105I	2	5077 6849
*Ecl*136I	1	216

*Eco*47III	2	1932 4795
*Eco*NI	3	3433 4923 5295
*Eco*RI	1	1383
*Eco*RV	2	1885 5660
*Esp*3I(*Bsa*I)	2	3120 8530
*Esp*I(*Bpu*1102I)	1	597
*Fsp*I	2	1960 6629
*Hin*dIII	3	885 1717 1729
*Hpa*I	2	1017 2272
*Kpn*I	2	2323 2934
*Msc*I	2	2204 3789
*Nco*I	1	3766
*Nde*I	1	8357
*Ngo*MI	2	4702 7294
*Nhe*I	2	1929 2875
*Not*I	3	6 5712 8596
*Nru*I	1	5208
*Nsi*I	2	684 1241
*Pfl*MI	2	196 1302
*Pme*I	1	420
*Ppu*MI	2	142 4339
*Pst*I	1	6608
*Pvu*I	1	6482
*Pvu*II	2	1600 4497
*Sac*I	1	216
*Sal*I	1	3312
*Sca*I	2	1360 6371
*Sph*I	1	4863
*Ssp*I	3	2806 6047 6983
*Stu*I	1	3395
*Tth*111I	1	8432
*Xba*I	1	2168

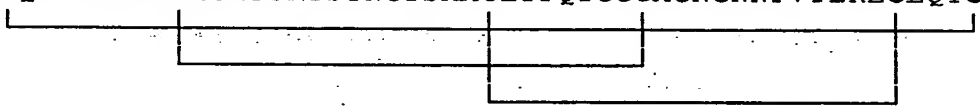
XcmI 1 711

Please replace Table 400 on page 112 with the following amended Table:

Table ~~400~~ 28: Amino-acid Sequence of ITI light chain (SEQ ID NO. 077)

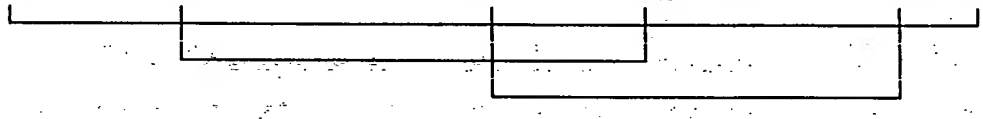
```
                    111111 111122
          12345 6789012345 678901
          avlpq eeegsgggql vteVtk

2222222233333333334444444445555555555666666666677777777
2345678901234567890123456789012345678901234567890123456
KEDSCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKECLQTC
```



```
77788
78901
rtvaa

                        11111111111111111111111111111111111
888888889999999999000000000011111111112222222222333333
234567890123456789012345678901234567890123456789012345
CNLPIVRGPCRAFIQLWAFDAVKGKCVLFPYGGCQGNGNKFYSEKECREYCGVP
```



```
                                          111111111111
                                          333344444444
                                          678901234567
                                          gdgdeellrfsn
```

ITI-D1 comprises residues 22-76 and optionally one of residue 77, residues 77 and 78, or residues 77-79.

ITI-D2 comprises residues 80-135 and optionally one of residue 79 or residues 78-79.

The lines under the sequences represent disulfides.

Please replace Table 602 on page 113 with the following amended Table:

TABLE ~~602~~ 30: Physical properties of hNE inhibitors derived from Kunitz domains

Protein	Parent	# Residues	Mol Wt	Predicted pI	K_D (pM)	k_{on} (10^6/M/s)	k_{off} (10^{-6}/s)
EPI-HNE-1	BPTI	58	6359	9.10	2.0	3.7	7.4
EPI-HNE-2	BPTI	62	6759	4.89	4.9	4.0	20.
EPI-HNE-3	ITI-D2	56	6179	10.04	6.2	8.0	50.
EPI-HNE-4	ITI-D2	56	6237	9.73	4.6	10.6	49.

The constants K_D and k_{on} above were measured with [hNE] = 8.47×10^{-10} molar; k_{off} was calculated from $k_{off} = K_D \times k_{on}$.

Please replace Table 603 on page 113 with the following amended Table:

TABLE ~~603~~ 31: SUMMARY OF PURIFICATION OF EPI-HNE-2

STAGE	Volume (ml)	Concentration (mg/ml)	Total (mg)	Activity (mg/A_{280})
HARVEST	3,300	0.70	2.31	< 0.01
30K ULTRA-FILTRATION FILTRATE	5,000	0.27	1.40	< 0.01
5K ULTRA-FILTRATION RETENTATE	1,000	1.20	1.20	0.63
AMMONIUM SULFATE PRECIPITATE	300	2.42	0.73	1.05
IEX pH6.2 ELUATE	98	6.88	0.67	1.03
EPI-HNE-3, LOT 1	50	13.5	0.68	1.04

Please replace Table 604 on page 114 with the following amended Table:

TABLE ~~604~~ 32: SUMMARY OF PURIFICATION OF EPI-HNE-3

STAGE	VOLUME (ml)	CONCENTRATION (mg/ml)	TOTAL (mg)	ACTIVITY (mg/A_{280})
HARVEST	3,100	0.085	263	nd
30K ULTRA-FILTRATION FILTRATE	3,260	0.055	179	0.007
FIRST IEX: pH6.2 ELUATE	180	0.52	94	0.59
AMMONIUM SULFATE PRECIPITATE	100	0.75	75	0.59
IEX pH9 ELUATE	60	1.01	60	0.59
EPI-HNE-3, LOT 1	26	1.54	40	0.45

Please replace Table 605 on page 115 with the following amended Table:

TABLE 605: K_I VALUES OF EPI-HNE PROTEINS FOR VARIOUS HUMAN SERUM SERINE PROTEASES

Enzyme	Inhibitor:			
	EPI-HNE-1	EPI-HNE-2	EPI-HNE-3	EPI-HNE-4
Human Neutrophil Elastase	2 pM	5 pM	6 pM	5 pM
Human Serum Plasmin	> 6 μM	>100 μM	>100 μM	>90 μM
Human Serum Kallikrein	>10 μM	>100 μM	>100 μM	>90 μM
Human Serum Thrombin	>90 μM	>100 μM	>100 μM	>90 μM
Human Urine Urokinase	>90 μM	>100 μM	>100 μM	>90 μM
Human Plasma Factor X_a	>90 μM	>100 μM	>100 μM	>90 μM
Human Pancreatic Chymotrypsin	~10 μM	~10 μM	~30 μM	~10 μM

Please replace Table 607 on page 116 with the following amended Table:

Table ~~607~~ 34: PEY-33 which produces EPI-HNE-2

Elapse Fermenter Time Hours:minutes	Cell Density (A_{600})	Activity in supernatent (mg/l)
41:09	89	28
43:08	89	57
51:54	95	92
57:05	120	140
62:43	140	245
74:45	160	360
87:56	170	473
98:13	190	656
102:25	200	678
109:58	230	710

Fermenter culture growth and EPI-HNE protein secretion by *P. pastoris* strains PEY-33. Time course is shown for fermenter cultures following initiation of methanol-limited feed growth phase. Increase in cell mass is estimated by A_{600}. Concentration of inhibitor protein in the fermenter culture medium was determined from measurements of hNE inhibition by diluted aliquots of cell-free CM obtained at the times indicated and stored at -20°C until assay.

Please replace Table 608 on page 117 with the following amended Table:

Table ~~608~~ 35: PEY-43 Which produces EPI-HNE-3

Elapse Fermenter Time Hours:minutes	Cell Density (A_{600})	Activity in supernatent (mg/l)
44:30	107	0.63
50:24	70	9.4
52:00	117	14.
62:00	131	28.
76:00	147	39.
86:34	200	56.
100:27	185	70.
113:06	207	85.

Fermenter culture growth and EPI-HNE protein secretion by *P. pastoris* strains PEY-43. Time course is shown for fermenter cultures following initiation of methanol-limited feed

growth phase. Increase in cell mass is estimated by A_{600}. Concentration of inhibitor protein in the fermenter CM was determined by assays of hNE inhibition by diluted aliquots of cell-free CM obtained at the times indicated and stored at -20°C until assay.

Please replace Table 610 on page 118 with the following amended Table:

Table ~~610~~ 36: Inhibitory properties of EPI-HNE-2

µl of EPI-HNE-2 solution added	Percent residual hNE activity
0.	101.1
0.	100.0
0.	100.0
0.	100.0
0.	100.0
0.	98.9
10.	82.9
20.	71.8
30.	59.5
40.	46.2
50.	39.2
55.	32.2
60.	22.5
65.	23.5
70.	15.0
75.	10.4
80.	8.6
85.	4.8
90.	1.4
95.	2.0
100.	2.5
120.	0.2
150.	0.2
200.	0.04

Please replace Table 611 on page 119 with the following amended Table:

Table ~~611~~ 37: hNE inhibitory properties of EPI-HNE-3

µl of EPI-HNE-3 solution added	Percent residual hNE activity
0.	101.2
0.	100.0
0.	100.0
0.	100.0
0.	100.0
0.	98.8
10.	81.6
20.	66.9
30.	53.4
40.	38.0
50.	27.6
55.	21.5
60.	13.0
65.	11.0
70.	7.9
75.	3.8
80.	3.3
85.	2.1
90.	1.8
100.	1.6
110.	0.8
120.	0.7
160.	0.6
200.	0.2

Please replace Table 612 on page 120 with the following amended Table:

Table ~~612~~ 38: pH stability of Kunitz-domain hNE inhibitors

Incubation pH	Percent Residual hNE Inhibitory Activity			
	EPI-HNE-1	EPI-HNE-2	EPI-HNE-3	EPI-HNE-4
1.0	102	98	97	98
2.0	100	97	97	100
2.6	101			
3.0	100	101	100	96
4.0	98	101	102	94
5.0	100			
5.5		99	99	109
6.0	100		103	99
6.5			99	100
7.0	93	103	103	93
7.5			87	109
8.0	96		84	83
8.5		104	68	86
9.4	100		44	40
10.0	98	102	27	34

Proteins were incubated at 37°C for 18 hours in buffers of defined pH (see text). In all cases protein concentrations were 1 μM. At the end of the incubation period, aliquots of the reactions were diluted and residual hNE-inhibition activity determined.

Please replace Table 620 beginning on page 121 to page 122 with the following amended Table:

Table ~~620~~ 39: Stability of hNE inhibitory proteins to oxidation by Chloramine-T

Table ~~620~~ 39	Percent Residual hNE-Inhibitory Activity					
Molar Ratio CHL-T: Inhibitor	EPI-HNE-1	EPI-HNE-2	EPI-HNE-3	EPI-HNE-4	α1 anti trypsin	SLPI
0	100	100	100	100	100	100
0.25		94				
0.29						93
0.30					97	
.48	102					
.50		102	97	100	85	
.59						82
.88						73
.95	100					
1.0		102	97	100	41	
1.2						65
1.4	98					
1.5		95				
1.9	102					
2.0		102				
2.1					7	
2.4						48
3.0			97	100		
3.8	94					
4.0		95				
5.0			94	100		
5.2					7	
5.9						18
9.5	95					
10.		98	97	104		
10.4					>5	
12.						15
19.	92					

Table ~~620~~ 39	Percent Residual hNE-Inhibitory Activity					
Molar Ratio CHL-T: Inhibitor	EPI-HNE-1	EPI-HNE-2	EPI-HNE-3	EPI-HNE-4	α1 anti trypsin	SLPI
30.			100	100		
50.			94	100		

Inhibitors were incubated in the presence of Chloramine-T at the molar ratios indicated for 20 minutes at RT. Oxidation reactions were quenched by adding methionine to a final concentration of 4 mM. Residual hNE-inhibition activity remaining in the quenched reactions is shown as a percentage of the activity observed with no added oxidant. Proteins and concentrations in the oxidation reactions are: EPI-HNE-1, (5 μM); EPI-HNE-2, (10 μM); EPI-HNE-3,(10 μM); EPI-HNE-4, (10 μM); API, (10 μM); and SLPI, (8.5 μM).

Please replace Table 630 on page 123 with the following amended Table:

Table 630: Temperature stability of EPI-HNE proteins

	Residual hNE Inhibitory Activity			
Temperature (°C)	EPI-HNE-1	EPI-HNE-2	EPI-HNE-3	EPI-HNE-4
0	97	101	96	100
23	100	103	105	103
37	100	97	99	98
45	103			
52		101	100	
55	99			98
65	94	95	87	
69				82
75	100			
80		101	79	
85	106			63
93		88	57	
95	64			48

Proteins were incubated at the stated temperature for 18 hours in buffer at pH 7.0. In all cases protein concentrations were 1 μM. At the end of the incubation period, aliquots of the reactions were diluted and residual hNE-inhibition activity determined.

Please replace Table 711 on page 124 with the following amended Table:

Table ~~711~~ 41: Mutations that are likely to improve the affinity of a Kunitz domain for hNE

Most Preferred

X18F;

[X15I(preferred), X15V];

Highly Preferred

[X16A(Preferred), X16G];

[X17F(preferred), X17M, X17L, X17I, X17L];

[{X19P, X19S}(equally preferred), X19K, X19Q];

X37G;

X12G;

Preferred

X13P;

X20R;

X21Y; X21W;

[X34V(preferred), X34P];

[X39Q, X39M];

[X32T, X32L];

[X31Q, X31E, X31V];

[X11T, X11A, X11R];

[X10Y, X10S, X10V];

[X40G, X40A];

X36G;

Please replace Table 720 on page 125 with the following amended Table:

Table ~~720~~ 42: M13_III_signal::Human_LACI-D2::mature_M13_III

DNA has SEQ ID NO. 078, amino-acid sequence has SEQ ID NO. 079. DNA is linear and *in vivo* it is double stranded.

Amino-acid sequence is of a protein that is processed *in vivo* by cleavage after Ala_{-1}; the entire gene encodes an amino-acid sequence that continues to give a functional M13 III protein.

```
  M   K   K   L   L   F
 -18 -17 -16 -15 -14 -13
|atg|aaG|aaG|ctt|ctc|ttc|
        |       |
        HindIII

  A   I   P   L   V   V   P   F   Y   S   G   A
 -12 -11 -10 -9  -8  -7  -6  -5  -4  -3  -2  -1
|gcc|att|cct|ctg|gtg|gta|cct|ttc|tat|tcc|ggc|gcc|
  | BstXI       | | KpnI |             | KasI |
  |     XcmI      |

  K   P   D   F   C   F   L   E   E   D   P   G
  1   2   3   4   5   6   7   8   9   10  11  12
|aag|cct|gac|ttc|tgc|ttc|ctc|gag|gag|gat|ccc|ggg|
                        | XhoI |        | XmaI |

  I   C   R   G   Y   I   T   R   Y   F
  13  14  15  16  17  18  19  20  21  22
|att|tgc|cgc|ggt|tat|att|acg|cgt|tat|ttc|
      | SacII|        | MluI |

  Y   N   N   Q   T   K   Q   C   E   R
  23  24  25  26  27  28  29  30  31  32
|tat|aat|aac|cag|act|aag|caa|tgt|gag|cgg|
                    | BsrDI| | BsrI |

  F   K   Y   G   G   C   L   G   N   M
  33  34  35  36  37  38  39  40  41  42
|ttc|aag|tat|ggt|ggt|tgc|cta|ggt|aat|atg|
                    | AvrII|

  N   N   F   E   T   L   E   E   C   K
  43  44  45  46  47  48  49  50  51  52
|aac|aac|ttc|gag|act|cta|gaa|gag|tgt|aag|
                  | XbaI |
  N   I   C   E   D   G   G   A   E   T   V   E   S
  53  54  55  56  57  58 100 101 102 103 104 105 106
|aac|ata|tgt|gag|gat|ggt|ggt|gct|gag|act|gtt|gag|tct|
  | NdeI |                          | DrdI         |
```

Ala_{101} is the first residue of mature M13 III.

Please replace Table 725 on page 126 with the following amended Table:

Table ~~725~~ 43: Synthetic *laci-d1* with sites for cloning into display vector

DNA has SEQ ID NO. 080, amino-acid sequence has SEQ ID NO. 081

```
    A   A   E   M   H   S   F   C   A   F   K   A   D
                1   2   3   4   5   6   7   8   9  10
5'-gcg|gcc|gag|atg|cat|tcc|ttc|tgc|gct|ttc|aaa|gct|gat|
   |EagI  |  | NsiI  |

    D   G   P   C   K   A   I   M   K   R
   11  12  13  14  15  16  17  18  19  20
  |gaC|ggT|ccG|tgt|aaa|gct|atc|atg|aaa|cgt|
    | RsrII |             | BspHI|

    F   F   F   N   I   F   T   R   Q   C
   21  22  23  24  25  26  27  28  29  30
  |ttc|ttc|ttc|aac|att|ttc|acG|cgt|cag|tgc|
                        | MluI  |

    E   E   F   I   Y   G   G   C   E   G   N   Q
   31  32  33  34  35  36  37  38  39  40  41  42
  |gag|gaA|ttC|att|tac|ggt|ggt|tgt|gaa|ggt|aac|cag|
     | EcoRI |                       | BstEII |

            N   R   F   E   S   L   E   E
           43  44  45  46  47  48  49  50
          |aac|cgG|ttc|gaa|tct|ctA|gag|gaa|
            |     | BstBI |  | XbaI |
            | AgeI |

    C   K   K   M   C   T   R   D   G   A
   51  52  53  54  55  56  57  58  59 101
  |tgt|aag|aag|atg|tgc|act|cgt|gac|ggc gcc
                              | KasI  |
```

Ala_{101} is the first residue of mature M13 III.

Please replace Table 730 on page 127 with the following amended Table:

Table ~~730~~ 44: LACI-D1 hNE LibraryDNA has SEQ ID NO. 082, amino-acid sequence has SEQ ID NO. 083

```
    A   A   E   M   H   S   F   C   A   F   K   A
                1   2   3   4   5   6   7   8   9
5'-gcg|gcc|gag|atg|cat|tcc|ttc|tgc|gct|ttc|aaa|gct|
   |EagI  |  | NsiI  |
                                            S
     T|N                                    T|N
  C|R K|R                                    I|M
  S|G S|A                                    Q|H
  Y|H E|G     H|R             F|L            L|P
  D|N  D   G  P|L  C  V|I A|G I|V  F  K|R  R
  10  11  12  13  14  15  16  17  18  19  20
 |NRt|RVS|ggT|cNt|tgt|Rtt|gSt|Ntc|ttc|MNS|cgt|

   C
  Y|W
  F|L  F   F   N   I   F   T   R   Q   C
  21  22  23  24  25  26  27  28  29  30
 |tDS|ttc|ttc|aac|att|ttc|acG|cgt|cag|tgc|
                         | MluI  |

      Q       Q                   Q
     L|P     L|P                 L|P
     T|K     T|K                 T|K
  L|Q V|I    V|E                 V|M         E|G
  E|V E|A  F  I|A  Y   G   G   C  E|A G|A  N  Q|R
  31  32  33  34  35  36  37  38  39  40  41  42
 |SWG|VHA|ttC|VHA|tac|ggt|ggt|tgt|VHG|gSt|aac|SRG|

          N   R   F   E   S   L   E   E
         43  44  45  46  47  48  49  50
        |aac|cgG|ttc|gaa|tct|ctA|gag|gaa|
          |     | BstBI |  | XbaI |
          | AgeI |

   C   K   K   M   C   T   R   D   G   A
  51  52  53  54  55  56  57  58  59  101
 |tgt|aag|aag|atg|tgc|act|cgt|gac|ggc gcc
                               | KasI  |
```

Variegation at 10, 11, 13, 15, 16, 17, 19, and 20 gives rise to 253,400 amino-acid sequences and 589,824 DNA sequences. Variegation at 31, 32, 34, 39, 40, and 42 gives 23,328 amino-acid and DNA sequences. There are about 5.9×10^9 protein sequences and 1.4×10^{10} DNA sequences.

Ala_{101} would be the first residue of mature M13 III.

Please replace Table 735 on page 128 with the following amended Table:

Table ~~735~~ 45: LACI-D2 hNE Library

DNA has SEQ ID NO. 084; amino-acid sequence has SEQ ID NO. 085

```
                                                                    P|H
                                                                    T|N
                                                               C|R K|R
                                                               S|G S|A
                                                               Y|H E|G
 G   A   K   P   D   F   C   F   L   E   E  D|N D|Q  G
-2  -1   1   2   3   4   5   6   7   8   9   10  11  12
|ggc|gcc|aag|cct|gac|ttc|tgc|ttc|ctc|gag|gag|NRt|VVS|ggg|
| KasI |                       | XhoI  |

                          I|N
H|R             F|L      Q|M
P|L             I|V      L|H       C
N|S             Y|H      K|P      F|L
I|T  C  V|I G|A N|D  F   T|R  R   Y|W  F
 13  14  15  16  17  18  19  20  21  22
|MNt|tgc|Rtt|gSt|NWt|ttt|MNS|cgt|tDS|ttc|

                                                Q|G
                                                L|P
                                                T|K
                                                V|I
                                             L|Q E|A
 Y   N   N   Q   A   K   Q   C  E|V  R
 23  24  25  26  27  28  29  30  31  32
|tat|aat|aac|cag|Gct|aag|caa|tgt|SWg|VNA|
                |      | BsrDI|
               | EspI   |

    Q|L                 Q|P
    P|T                 T|K             R|G
    V|E                 V|M             K|E
    I|A                 E|A             L|Q
 F   K   Y   G   G   C   L  G|A  N  M|V
 33  34  35  36  37  38  39  40  41  42
|ttc|VHA|tat|ggt|ggt|tgc|VHG|gSt|aat|VBg|

 N   N   F   E   T   L   E   E   C   K
 43  44  45  46  47  48  49  50  51  52
|aac|aac|ttc|gag|act|cta|gaa|gag|tgt|aag|
                  | XbaI |

 N   I   C   E   D   G   G   A   E   T   V   E   S
 53  54  55  56  57  58 100 101 102 103 104 105 106
|aac|ata|tgt|gag|gat|ggt|ggt|gct|gag|act|gtt|gag|tct|
  | NdeI |                        |  DrdI          |
```

6.37×10^{10} amino acid sequences; 1.238×10^{11} DNA sequences

Please replace Table 790 on page 129 with the following amended Table:

Table ~~790~~ 46: Amino acids preferred in hNE-inhibiting Kunitz domains	
Position	Allowed amino acids
5	C
10	YSV, (NA)
11	TAR, (QP)
12	G
13	P, (VALI)
14	C
15	IV
16	AG
17	FM, ILV(A)
18	F
19	PS, QK
20	R
21	YW, (F)
30	C
31	QEV, (AL)
32	TL, (PSA)
33	F
34	VP
35	Y
36	G
37	G
38	C
39	MQ
40	G,A
41	N highly preferred
42	G preferred, A allowed
45	F
51	C
55	C

Please insert after Table 20 (formerly Table 219), page 89 the following Table:

TABLE 21

ITI-D1-derived hNE Inhibitors

WEAK (K_D > 10^{-8} **M**)

```
                1    1     2   2    3    3    4    4    5    5
    1...5....0....5.....0...5....0....5....0....5....0....5...
1.  KEDSCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

MODERATE (10^{-8} > RD 22 10^{-9})

```
2.  KEDSCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
3.  RPDFCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

STRONG (10^{-9} ≥ KD > 10^{-11} D)

```
4.  RPDFCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
5.  RPDFCQLGYSTGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
6.  KEDFCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
7.  KPDSCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
8.  RPDFCQLGYSAGPCIGMFSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

VERY STRONG (K_D < 10^{-11} **M**)

```
9.  RPDFCQLGYSAGPCVAMFPRYFYNGTSMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
10. RPDFCQLGYSAGPCVAMFPRYFYNGASMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
11. RPDFCQLGYSAGPCVAMFPRYFYNGTSMACETFVYGGCMGNGNNFVTEKDCLQTCRGA
12. RPDFCQLGYSAGPCVGMFSRYFYNGTSMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
```

Residues shown underlined and bold are changed from those present in ITID1

Sequences Key:

1.	ITI-D1	SEQ ID NO. 008
2.	ITI-D1E7	SEQ ID NO. 009
3.	BITI	SEQ ID NO. 030
4.	BITI-E7	SEQ ID NO. 010
5.	BITI-E7-1222	SEQ ID NO. 012
6.	AMINO1	SEQ ID NO. 015
7.	AMINO2	SEQ ID NO. 016
8.	MUTP1	SEQ ID NO. 014
9.	BITI-E7-141	SEQ ID NO. 011
10.	MUTT26A	SEQ ID NO. 018
11	MUTQE	SEQ ID NO. 017
12	MUT1619	SEQ ID NO. 013

Please insert after Table 21 (formerly Table 220), page 89 the following amended Table:

TABLE 22

Same sequences as in Table ~~220~~ 21 showing only changes (and Cysteines for alignment).

WEAK (K_D > 10^{-8} M)

```
                1    1     2   2    3    3    4    4    5    5
    1...5....0....5.....0...5....0....5....0....5....0....5...
1.  KEDSCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

MODERATE (10^{-8} > RD 22 10^{-9})

```
2.  ---C---------CVA-FP----------C-------C------------C---C---
3.  RP--C---------C---------------C-------C------------C---C---
```

STRONG (10^{-9} > KD > 10^{-11}D)

```
4.  RP--C---------CVA-FP----------C-------C------------C---C---
5.  RP--C-----T--CVA-FP----------C-------C------------C---C---
6.  ---FC---------CVA-FP----------C-------C------------C---C---
7.  -P--C---------CVA-FP----------C-------C------------C---C---
8.  RP-FC---------CI--FP----------C-------C------------C---C---
```

VERY STRONG (K_D < 10^{-11} M)

```
9.  RP-FC---------CVA-FP----------CQ--V---C------------C---C---
10. RP-FC---------CVA-FP------A---CQ--V---C------------C---C---
11. RP-FC---------CVA-FP----------C---V---C------------C---C---
12. RP-FC---------CV---F----------CQ--V---C------------C---C---
```

Residues shown underlined and bold are changed from those present in ITID1.

REMARKS

Applicants present this amendment in response to the Examiner initiated telephone interview with Applicants' representatives Michael Siekman and Marie Aucoin. Applicants have amended the specification as requested to renumber the Tables consecutively as they appear in the text. Applicants have amended the Tables to reflect the amended Table numbers. Applicants have inserted Table 220 (now Table 21) and Table 221 (now Table 22) from U.S. Patent No. 5,663,143 which is incorporated by reference. Support for this insertion is found on page 1, lines 21-22 and page 21, lines 11-14. Applicants have added the sequence identifiers for each of the sequences in Table 5 (formerly Table 13) and corrected the sequence identifiers for the sequences in Tables 12-13 (formerly Tables 207-208). Support is found in the sequence listing. In the paragraphs that originally contained underlined text, Applicants have used double-underlining to distinguish the added text from the original text. No new matter has been added.

The Examiner also indicated the priority claims for this application was confusing. As Applicants' representatives discussed with the Examinrer, Applicants have amended the application to clearly claim priority back to Application Serial No. US 08/133,031.

Applicants submit herewith a substitute sequence listing to replace the sequence listing submitted June 7, 2002. As discussed with Examiner Moore on March 16, 2005, Applicants have corrected the original sequence list in the following way. The title was corrected to correct a typographical error ("nHE" was replaced with "HNE") and the docket number updated to reflect our docket number. The priority information was updated to reflect the corresponding priority information in the amended paragraph of the specification. A typographical error in SEQ ID NOs:1 and 2 was corrected ("mautre" was replaced with "mature"). The eleven sequences disclosed in the specification that were not included in the original sequence list were added and the corresponding paragraphs updated to include the sequence identifiers. SEQ ID NOs:103, 104, 106, 109 and 123 were corrected to replace "Glx" at residue 1 with Xaa, wherein Xaa is Glu or Gln. Support for this is found in Table 5 (formerly Table 13) wherein the first amino acid residue is disclosed as "z" which represents Glu or Gln (see MPEP § 2422, Table 1). SEQ ID NOs:82 and 84 were corrected to include each of the undefined nucleotide residues, for

example residue 38 in SEQ ID NO:82 is disclosed as "r" which represents adenine or guanine (see MPEP § 2422, Table 1). Support for this is found in the specification on page 127.

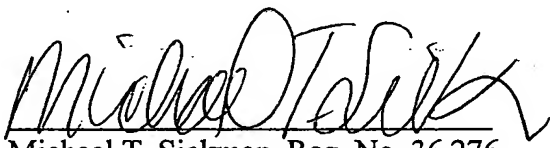
As the Examiner requested, Applicants file herewith a Terminal Disclaimer over U.S. Patent No. 5,663,143.

CONCLUSION

Applicants believe this amendment puts the claims in condition for allowance. A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance to resolve an remaining issues.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
Arthur C. Ley et al., Applicant(s)

By: 

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Docket No.: D0617.70005US01
Date: March 17, 2005
xxNDDxx

Examiner-Initiated Interview Summary

Application No.	Applicant(s)	
10/038,722	LEY ET AL.	
Examiner	**Art Unit**	
William W. Moore	1652	

All Participants: **Status of Application:** *Election*

(1) *William W. Moore, Examiner.* (3) _____.

(2) *Mr. Michael T. Seikman, Applicant's Counsel.* (4) _____.

Date of Interview: *2/20/ & 3/16/2005* **Time:** *11:00AM*

Type of Interview:
- ☒ Telephonic
- ☐ Video Conference
- ☐ Personal (Copy given to: ☐ Applicant ☐ Applicant's representative)

Exhibit Shown or Demonstrated: ☐ Yes ☐ No
If Yes, provide a brief description:

Part I.

Rejection(s) discussed:

Claims discussed:
Original claims 1-31 and 33-36

Prior art documents discussed:

Part II.

SUBSTANCE OF INTERVIEW DESCRIBING THE GENERAL NATURE OF WHAT WAS DISCUSSED:
See Continuation Sheet

Part III.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview directly resulted in the allowance of the application. The examiner will provide a written summary of the substance of the interview in the Notice of Allowability.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview, since the interview did not result in resolution of all issues. A brief summary by the examiner appears in Part II above.

(Examiner/SPE Signature) (Applicant/Applicant's Representative Signature – if appropriate)

Continuation of Substance of Interview including description of the general nature of what was discussed: In the telephonic interview initiated by the examiner on 10 February 2005, Applicant's counsel requested rejoinder of methods of claims 33-36 should the elected species be found allowable and the examiner noted that several of the original claims may not read on the elected species. It was agreed that, in view of the Election received 18 January 2005, a Terminal Disclaimer over claims of US 5,663,143 and amendments to the specification correcting, at least, the continuing data in the first two paragraphs at page 1 of the of the specification would be necessary. In the telephonic interview of 16 March 2005, the revision of Table enumeration and SEQ ID NO numeration in a Preliminary Amendment was discussed.